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CONTENTS

Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustaceae: Brachyura) from Visakhapatnam coast
C A Nageswara Rao, K Shyama Sundari and K Hanumantha Rao 1

The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths *K S S Nair and V V Sudheendrakumar* 7

Functional morphology, anatomy and histology of the digestive organs of the catfish, *Plotosus canius* Hamilton *M Sinha* 23

Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats
P A Dechamma, S Kamath and P S Shetty 45

Effect of ethylestrenol on growth and food utilization in *Channa striatus*
V Arul 51

Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) *R Baskaran and H Md Mohideen* 59

Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* Hamilton
M Peer Mohamed and M N Kutty 67

Distribution and abundance of prawns in the freshwater habitats of Bangalore, South India
K V Anantha Raman, S Ravichandra Reddy and Katre Shakuntala 77

Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours *Mohd Idris and Ishwar Prakash* 89

A study on the effects of certain abiotic factors on the activity of *Adoretus epipleuralis* Arrow (Coleoptera: Scarabaeidae) *Rohtash Chand* 97

Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) *K M Renukaradhya and T J Varghese* 103

Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants *R Velayudhan and R S Annadurai* 109

Sensillar diversity, density and distribution during post-embryonic development of *Cyrtacanthacris ranacea* Stoll and their role in feeding *T N Ananthakrishnan, N Suresh Kumar and K P Sanjayan* 11

Composition and variations in the abundance of zooplankton in the Hooghly estuary, West Bengal, India *S K Sarkar, B N Singh and A Choudhury* 12

Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera) *G K Manna and S K Dey* 13

Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton *M Sriramulu and K N Mehrotra* 14

Hematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters) *M A Haniffa, A G Murugesan and M Porchelvi* 15

Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) *K Venkataraman, M Kesary and S Krishnaswamy* 16

Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca) *N Munuswamy and T Subramoniam* 17

Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region *D Ghosh, P K Medda and S Chakrabarti* 18

Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae) *B Chandrika and N K Balasubramonian* 18

Wings and wing-microsculpturing in the termite family Indotermitidae (*Indotermes*, Isoptera), and their bearing on phylogeny *M L Roonwal and N S Rathore* 19

Competition studies in *Drosophila*: Relative fitness and adaptedness of six closely related species *H A Ranganath and N B Krishnamurthy* 19

Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera) *A C Bhardwaj* 20

Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid fish, *Oreochromis mossambicus* (Peters), with special reference to swimming speed *N Sukumaran* 21

Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons *Shilla Chatterjee, Dhananjay Pal and Ashok Ghosh* 22

Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål) *Kasi Viswanathan and M B Kalode* 22

Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) *E T Haridass* 23

Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions *K Venkataraman and S Krishnaswamy* 24

Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes *A Triveni and P R Rao* 255

Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats *B B Kaliwal, R Nazeer Ahamed and M Appaswamy Rao* 263

Some observations on the biology of periwinkle *Littorina scabra scabra* (Linne 1758) *S Maruthamuthu and R Kasinathan* 269

Evolution and biogeography of the Indian genera of the family Ariidae *K C Jayaram and J R Dhanze* 279

Production and utilization of frogs: an ecological view *T J Pandian and M Peter Marian* 289

Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) *M S M Christopher and S Mathavan* 303

Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries *A H Parulekar, Z A Ansari and B S Ingole* 325

Biological activity of earthworm casts: An assessment of plant growth promoter levels in the casts *R V Krishnamoorthy and S N Vajranabhaiah* 341

The physico-chemical environment and the plankton of managed ponds in Haryana, India *R N Singhal, Swarn Jeet and R W Davies* 353

Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India) *D N Saksena and N Kulkarni* 365

Effect of artificial aeration on the growth and survival of Indian major carps *M M Vijayan and T J Varghese* 371

Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) *A Mini and V K K Prabhu* 379

Prediction of absorption efficiency from food nitrogen in amphibians *T J Pandian and M Peter Marian* 387

Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita* *Ch Venkata Rao, A Mani and P Kameswara Rao* 397

Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* *B B P Gupta and J P Thapliyal* 403

Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina* *J Muthukrishnan, M Senthamizhselvan and S Mohan* 411

Laboratory evaluation of bait base for the control of sand coloured rat, *Rattus gleadowi* *Md Idris, A P Jain and B K Soni* 417

On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) *R J Lazarus and P Sita Rami Reddy* 423

Feeding ecology of the large grey babbler *Turdoides malcolmi* *H S Toor and Manjit S Saini* 429

Distribution and zonation of shore crabs in the Pulicat lake
D R Joel, P J Sanjeeva Raj and R Raghavan 437

Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta-Heteroptera) *E T Haridass* 447

Growth and metabolism of the catfish *Clarias batrachus* (Linn) fed with different experimental diets
B Venkatesh, A P Mukherji, P K Mukhopadhyay and P V Dehadrai 457

Trypanosoma evansi infection and the influence of some hormones on haematological indices in albino rats
M Lemalatha, L Narasimha Rao and M Kameswari 463

Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures
Mrinal Ghosh and Amalesh Choudhury 469

Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city with a note on mite infestation
B Ayyachamy Daniel, Cheriyan Thomas, K Kalyanaraman and R S Prasad 475

Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records *Kenneth A Spencer* 487

Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions *K Venkataraman and S Krishnaswamy* 509

Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson) *S Ajmalkhan, K Rajendran and R Natarajan* 515

In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure
M Srinivasulu Reddy and K V Ramana Rao 525

Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure
M Srinivasulu Reddy and K V Ramana Rao 533

Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos)
V Uma Devi, Y Prabhakara Rao and D G V Prasada Rao 539

Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India
P S Sasikumar, Prema Suryanarayanan, Cheriyan Thomas, K Kalyanaraman and R S Prasad 549

Adaptedness of five closely related strains of *Drosophila* to media containing molasses or fructose *A Ushakumari and H A Ranganath* 557

Seasonal effect on certain developmental stages of *Tricholyga bombycis* Beck. (Diptera: Tachinidae) *Pradip Kumar and M S Jolly* 561

Visual capacity of marine teleost *Nemipterus japonicus* (Bloch) *S Raveendran and H Md Mohideen* 567

Time and energy budgets of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) *D P Abrol* 579

Induction and genotypic verification of recombinants in males of *Drosophila melanogaster* *Gurbachan S Miglani and Vindhya Mohindra* 587

A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) *Mahavir Gupta* 595

Ontogeny of play behaviour in free-ranging rhesus macaques (*Macaca mulatta*) *Rajbir Singh* 605

Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes *P H Pathak and S S Krishna* 613

Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio* *Sumit Homechaudhuri, Tapan Pandit, Subhas Poddar and Samir Banerjee* 617

Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy *S L Choubisa* 623

Growth and population dynamics of the Indian white prawn *Penaeus indicus* H M Edwards from Kakinada *S Lalitha Devi* 629

Prediction of assimilation efficiency of lepidopterans *T J Pandian and M Peter Marian* 641

Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) *M Balakrishnan, V E Sokolov and O A Basurmanova* 667

Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) *K P Shahi and S S Krishna* 677

Occurrence of gastric absorption in the cichlid fish, *Sarotherodon mossambicus* *M Narayanan and S V Job* 685

Effect of an insect growth regulator on the growth and development of the tobacco caterpiller, *Spodoptera litura* Fabricius *G T Gujar and K N Mehrotra* 689

Heliothis species and their natural enemies, with their potential for biological control *C S Pawar, V S Bhatnagar and D R Jadhav* 695

Testicular damage in rats fed on irradiated diets *A K S Kushwaha and S S Hasan* 705

Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) *C A Nageswara Rao, K Shyama Sundari and K Hanumantha Rao* 713

Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch)
K R S Sambasiva Rao and K V Ramana Rao 719

Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation *G K Kulkarni, A Bhaskar Rao and C S K Anand* 723

Effects of ecological factors on the performance of rodenticides in sugar cane
V R Parshad, Nafis Ahmad and G Chopra 729

Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros* *R Jeyaraj, T Thangaraj, C A Vasuki and M Aruchami* 739

Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants *R S Annadurai and R Velayudhan* 745

Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device
Prakash Nautiyal, A S Saklani and H R Singh 751

Factors affecting pollination activity of *Megachile lanata* lepel
D P Abrol and R P Kapil 757

Subject index 771

Author index 784

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Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast*

C A NAGESWARA RAO**, K SHYAMA SUNDARI and
K HANUMANTHA RAO
Department of Zoology, Andhra University, Waltair 530 003, India
**Zoological Survey of India, Hillpatna, Berhampur 760 005, India

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Abstract. Reproductive cycle of the crab *Ocypoda macrocera* has been studied employing the gonad index method. Breeding period in this crab is found to be a prolonged one extending from March–September with small intermittent peaks and falls. Salinity is found to have a close relationship with the breeding cycle as lower salinities during October–January coincided with the absence of reproductive activity. Male crabs have not shown any clear picture in their gonad index in relation to breeding period and male gametes are found round the year.

Keywords. *Ocypoda macrocera*; reproductive cycle; gonad index; tropical marine invertebrates; salinity.

1. Introduction

Marine invertebrates are found to show different patterns in their reproductive behaviour and the reproductive biology of marine invertebrates have been studied extensively (Benett and Giese 1955; Farmanfarmain *et al* 1958; Rahaman 1966, 1967; Knudsen 1964; Chandran 1968; Pillay and Nair 1971, 1973; Nagabushanam and Kulkarni 1977). The relation of gonad weight to the body weight as a factor in the determination of the breeding periodicities in the marine invertebrates has been well established and the reproductive cycles of crustaceans based on gonad index method was studied by several authors (Subrahmanyam 1963; Rahaman 1967; Pillay and Nair 1971; Diwan and Nagabushanam 1974). The gonad index method involves the determination of the ratio of gonad weight to body weight and it is evident that gonad index of animals with immature or spent gonads will be lesser while the gonad index of ripening and mature gonads will be high.

Various patterns and differences in the timing of the reproductive cycles among marine invertebrates from Indian coasts were reported and environmental factors were attributed as a major factor to them. As there are scanty reports on the reproductive biology of marine invertebrates from Visakhapatnam coast, the common shore crab *Ocypoda macrocera* has been taken for such study.

2. Materials and methods

Fortnightly samples of *O. macrocera* were collected from Lawson's Bay and palm beach localities of Visakhapatnam coast. Live crabs were brought to the laboratory and kept in glass jars containing sea water. At the initial stages of the study, samples of the crabs were dissected to ascertain average size range of the maturing forms; while collecting samples, care was taken to collect only the crabs within the size ranges of their sexual maturity and in intermoult stage.

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*Part of the Ph.D. thesis (CANR) approved by the Andhra University, Waltair.

In the laboratory, the specimens were thoroughly dried with filter paper to remove the outer water content. Size measurements viz. carapace length and width were taken and later the weight of each crab was made. The crabs were dissected out and the net weight of the gonads of all the crabs were taken separately. The gonad index was calculated as wet weight of gonad divided by wet weight of the animal and this fraction multiplied by 100. A minimum of 10 crabs for each sex were taken during every sampling and the mean values for each sampling period for both males and females were calculated. Thus the mean gonad index for all the months of a year was obtained. The sampling was taken from May 1981–August 1982 and during sampling at every time small pieces of both the gonads were fixed and later sectioned for histological observations.

3. Results and observations

The histological studies on the gonads revealed that female crabs measuring above 2·2 cm and male crabs of 2·1 cm carapace width are found to be mature. The carapace width range, range of gonad index and mean gonad indices of female and male crabs for all the months from June 81–May 82 are shown in tables 1 and 2. In the female crabs, during the period from October–January minimum gonad indices of 0·71, 0·45, 0·69 and 0·79 were recorded. Ovarian activity started in the month of February as indicated by the higher gonad index (1·32) and also high individual gonad index (1·77) in comparison to January. Increase in the gonad index continued and higher gonad

Table 1. *O. macrocera*-carapace width range, range and mean of gonad indices of female crabs for different months during 1981–82.

Year and month	Carapace width range Cm	Range of gonad index	Mean of gonad index
1981			
June	2·4–3·3	0·34–4·23	2·144 ± 0·906
July	2·2–2·6	0·66–8·02	3·945 ± 2·630
August	2·2–2·7	0·73–5·11	2·159 ± 1·795
September	2·3–2·9	0·40–9·80	2·659 ± 3·164
October	2·2–2·7	0·38–0·93	0·713 ± 0·262
November	2·5–3·0	0·15–0·62	0·456 ± 0·259
December	2·5–2·9	0·42–0·75	0·692 ± 0·139
1982			
January	2·4–3·0	0·25–0·92	0·794 ± 0·424
February	2·7–2·9	0·92–1·77	1·320 ± 0·411
March	2·5–3·0	0·56–10·96	2·557 ± 4·708
April	2·5–2·9	0·40–7·33	2·929 ± 2·146
May	2·4–3·1	1·10–10·42	2·586 ± 1·560

Table 2. *O. macrocera*-carapace width range, range and mean of gonad indices of male crabs for different months during 1981–82.

Year and month	Carapace width range Cm	Range of gonad index	Mean of gonad index
1981			
June	2·3–2·8	0·63–1·28	0·836 ± 0·150
July	2·1–2·9	0·60–0·90	0·754 ± 0·120
August	2·2—2·8	0·678–0·976	0·818 ± 0·274
September	2·2–2·4	0·356–0·932	0·452 ± 0·258
October	2·2–2·4	0·550–0·836	0·660 ± 0·152
November	2·4–2·8	0·360–0·890	0·626 ± 0·212
December	2·4–2·9	0·260–0·754	0·444 ± 0·134
1982			
January	2·5–2·8	0·446–0·754	0·598 ± 0·154
February	2·5–2·9	0·794–1·156	0·842 ± 0·048
March	2·2–2·7	0·953–1·128	1·031 ± 0·089
April	2·6–2·7	0·473–0·752	0·615 ± 0·139
May	2·2 –2·8	0·618–0·871	0·790 ± 0·116

index values are found up to September though maximum gonad index 3·94 was noted in July.

It is evident from the figure 1 that the gonad indices from March–September remained nearly at uniform level though minor peaks during April and July and slight falls in June and August were noted. The reproductive activity started in March and continued up to September as evident from the higher mean gonad indices (2·55, 2·65) and also higher individual gonad indices (10·96, 9·80) were found in March and September respectively. The sudden fall in the mean gonad index and also individual gonad index from October–January indicate the cessation of reproductive activity during this period. Histological observations of ovaries coincided with the above results showing developing oocytes during February–September and their absence from October–January.

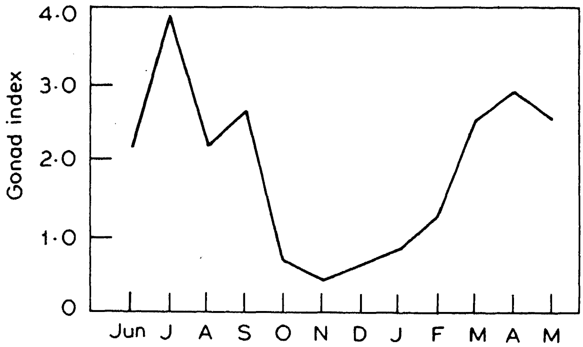

Figure 1. Seasonal variation in the gonad index of female crab *O. macrocera* during 1981–1982.

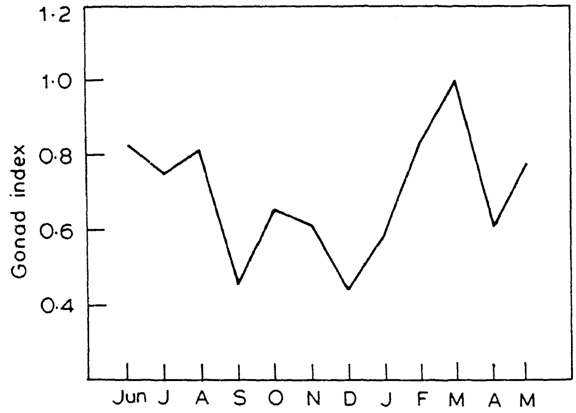

Figure 2. Seasonal variation in the gonad index of male crab *O. macrocera* during 1981–1982.

The gonad index values of the male crabs has not shown peaks or significant falls in its activity. It appears from the results shown in the table 2 and figure 2 that the gonad index of male crabs remained at nearly uniform level during most of the year except for a rise in March and fall in September and December. Maximum gonad index of 1·03 in March and minimum of 0·44 in December is noted. The results suggest more prolonged and frequent activity of the testis; During March not only maximum mean gonad index but also higher individual gonad index value was recorded, showing higher testicular activity at the beginning of breeding season. However histological observations of testis revealed the presence of developing male gametes even during minimum gonad index periods.

4. Discussion

The results clearly showed that the breeding period of *O. macrocera* is a prolonged one extending for about 7 months with higher activity during certain months. Female crabs have shown peak activity during April, July and September as evident from high gonad indices recorded (2·92, 3·94, 2·65). Though male crabs also have shown higher values of gonad indices during certain months of the active breeding period its value in assessing reproductive cycle is insignificant as testis is very minute in size; Rajabai Naidu (1955) reported female crabs of *O. macrocera* measuring 2·2 cm carapace width are mature, and could collect only one example of ovigerous crab during a period of 3 years in the month of August. In the present study also only 3 ovigerous crabs are collected in March. Therefore the data on the incidence of ovigerous females could not be combined with gonad index data in the study of the reproductive cycle for this crab.

The prolonged breeding period from March–September is interspersed with minor peaks and falls. The fall of the gonad index values in June and August during the reproductive period is probably due to the occurrence of majority females which are in the process of recuperation of their gonads after the release of the first brood and some females with spent ovaries. Development of new brood within a period of 15–20 days after the first brood has been reported in *Uca annulipes* (Pillay and Nair 1973).

Differences in the breeding behaviour and reproductive seasons are reported in several crustaceans from Indian coasts. In *U. annulipes* the breeding period extending from July–April with 3 distinct periods of gonadal activity was reported (Pillay and Nair 1971). In *Portunus pelagicus* from Madras coast a continuous breeding activity was noticed from November–June (Rahaman 1967) while in the same crab from west coast of India, a lengthy extended breeding period from August–April was reported (Pillay and Nair 1971). In the crabs inhabiting intertidal sands and muddy estuarine breaches of west coast, breeding period extending from September–April and in the swimming crabs a continuous breeding activity round the year was noted (Pillay and Nair 1973). The breeding pattern of *O. macrocera* from Visakhapatnam coast resembles to some extent with those of other intertidal crabs, by its extended breeding activity with intermittent peaks.

The variations in breeding pattern in the tropical marine invertebrates has been attributed to changes in salinity and temperature. The coincidence of maximum breeding activity with higher salinity is reported in the pearl oyster *Margritifera vulgaris* by Malps (1933). Similarly Panikkar and Aiyar (1939) and Pillay and Nair (1971) also indicated that the sudden changes to low salinities trigger off the reproductive activity in animals.

The breeding behaviour of *O. macrocera* agrees well with the earlier reports on the influence of salinity on reproductive cycle as periods of high salinities coincided with breeding activity and its absence during low salinities. Sunita Devi (1984) reported salinities of 33·63–35·97‰ from February–September and 23·02–29·97‰ from October–January in the near shore waters of Visakhapatnam during 1981–1982.

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The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths

K S S NAIR and V V SUDHEENDRAKUMAR
Division of Entomology, Kerala Forest Research Institute, Peechi 680 653, Kerala, India

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Abstract. In teak plantations at Nilambur, Kerala, *Hyblaea puera* Cramer (Lepidoptera, Hyblaeidae) caused one or two waves of epidemic defoliations between late April and July, followed in some years by isolated, lighter defoliation between August and November. The insect was absent at other periods, although with a 3-week life cycle it can theoretically pass through several generations per year. The temporal and spatial distribution of infestation and certain behavioural characteristics of populations gave evidence of short-range migration of newly emerged moths. In a model proposed for population dynamics of *H. puera*, no diapause occurs and a residual, non-migratory population exists in natural forests during the non-epidemic period. With the onset of general flushing of teak in February–March, the population starts building up and when a critical density is reached, migratory behaviour is triggered. Migration facilitates exploitation of new food sources and escape from larval parasites. Generally, after one or two epidemics, the population declines due to leaf maturity, natural enemies and density-dependent food depletion. This cycle of ups and downs, with attendant transition between migratory and non-migratory phases is repeated every year. If some steps of the proposed model are confirmed by further study, simple methods could be devised to manage this serious pest of teak.

Keywords. Teak defoliator; *Hyblaea puera;* migration; defoliation dynamics; population dynamics; limiting factors.

1. Introduction

Hyblaea puera Cramer (Lepidoptera, Hyblaeidae) is a well-known pest of teak in India. Though it has been shown that theoretically the insect can pass through at least 14 generations per year, in teak plantations, it is abundant only at certain times of the year—at Nilambur, for example, from April to June and sometimes also from August to September (Beeson 1941). Several reasons have been suggested for this difference between theory and observations. Aestivation in summer and hibernation in winter (in northern India) have been suspected by Chatterjee (1932) and Beeson (1941). Nair (1980) stressed the role of natural control factors including climate and natural enemies. Recently, Vaishampayan and Bahadur (1983) recorded sudden appearance of large numbers of moths in light traps at Jabalpur, Madhya Pradesh in July–August after "absolute absence" for a period of at least 6 months. They concluded that the insect does not breed in the area on any host plant during a period of about 9 months and suggested that they either migrate or enter diapause as pupae or adults. In a subsequent study, Vaishampayan *et al* (1984) showed that in moths caught during the early part of the season, the proportion of females, the maturity level of the ovary and the fat body

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KFRI Scientific Paper No. 77.

content of females were higher than in those caught later, indicating the occurrence of a migratory phase—"immigration in July and migration in August".

As part of a study on the effect of defoliation on wood increment, we recorded the intensity and frequency of defoliation caused by *H. puera* over a period of 4 years, in study plots laid out within teak plantations. These data, together with some fortuitous field observations which led to further inquiry into the spatial and temporal distribution of infestation, gave new insights into the population dynamics and migratory behaviour of this species. The new knowledge also suggests a novel strategy for management of the pest.

2. Methods

Three kinds of observations were made—(i) detailed study of annual defoliation trends in teak plantations at Nilambur, (ii) observations on infestation characteristics at Peechi, and (iii) survey of the distribution of infestation over selected tracts in the Western Ghats. In addition, field notes on infestation made over several years, in different parts of Kerala were utilized.

2.1 *Study of annual defoliation trends*

This study was made in Karulai Range of Nilambur Forest Division in plots laid out in selected teak plantations situated within a large teak plantation area ($> 10\ \text{km}^2$). There were 4 plots, 2 each in two plantations of the same age (considered as blocks), separated by a distance of about 4 km. These plots formed the untreated controls of an experiment designed to study the effect of defoliation on wood increment using 12 similar plots. The plantations were 5-year-old when observations began. The area between these plantations (blocks) was covered by older teak plantations. Each plot, $400\ \text{m}^2$ in area, had an average of about 35 trees (originally planted at a spacing of 2 m × 2 m; half the number of trees were thinned in the 4th year as per standard silvicultural practice). The location of each tree was marked in a plot chart and each tree was visually scored for defoliation, at fortnightly intervals for 4 years from 1979–1982. Scores were assigned to indicate the gross percentage of tree foliage lost due to defoliation: score 1 represented loss of 0–5 % of the foliage 2:6 to 25 %; 3:26 to 50 %; 4:51 to 75 %; 5:76 to 95 %; and 6:96 to 100 %. Using the midpoint of each range, the scores were converted to percentage foliage loss and the mean loss for each plot was calculated for each observation date. Defoliation curves were prepared using these values. In addition to this scoring, qualitative data on the species of defoliator involved, the stage of its development, etc., were recorded for each plot. According to the scoring system used, even when there was no defoliation, the plots would receive score 1, indicating loss of 2·5 % (mid point of the range) of the foliage. This system was followed since grazing by at least a few insects like grasshoppers and beetles could be expected most of the time. For purposes of this study, a mean leaf loss of 2·5 % or below was taken as zero, unless the presence of *H. puera* larvae was indicated by field notes. No other insect caused measurable defoliation during the periods when *H. puera* was prevalent and it was therefore possible to separate the defoliation curve for *H. puera*. To describe the annual defoliation trend, the 'growth year', covering the 12-month period commencing on 1 April and ending on 31 March, is used, instead of the calendar year.

2.2 *Observations on infestation characteristics*

These observations started fortuitously in April 1984 when one of us noticed a sudden build-up of *H. puera* infestation on teak trees surrounding his residential quarters within the Kerala Forest Research Institute Campus at Peechi. Teak was the dominant tree species in this belt of degraded natural forest, and scattered around the house were about 30 trees above 75 cm in girth and a large number of naturally regenerating teak saplings, less than 2 m high. Following the heavy infestation, clear-cut evidence of moth emergence was obtained from moths attracted to normal domestic lighting. Trapping was not feasible because the moths were attracted to all the domestic lights and it was neither possible to set up traps on all of them nor to regulate the domestic use of lights over the entire period of moth emergence. Following moth emergence, the trees and saplings around the house were kept under general observation for 11 months to discover new infestation; close examinations were made, usually, every Sunday.

2.3 *Spatial distribution of infestation*

Since the characteristics of infestation suggested migration of moths, a ground survey was conducted soon after the first population build-up at Peechi in 1984 to study the pattern of spread of infestation. A large extent of roadside teak plantations along the Western Ghats in Kerala and part of Karnataka, as well as some strips of teak-bearing natural forests were covered within a period of 8 days. Old as well as current infestations were easily detected from a distance and ground checks were made for confirmation.

3. Results

3.1 *Annual defoliation curves*

Figure 1 shows the defoliation caused by *H. puera* during 1979–1982 in the 4 plots. This defoliation curve is a faithful representation of the population curve of *H. puera* larvae, except that when the trees are totally defoliated, any increase in the population of larvae will not be reflected. Thus no comparison between years is possible, with respect to the population size at the time of total defoliation. With this restriction, the defoliation curve can be used to study the population dynamics of the insect. A study of the defoliation curves and simultaneously recorded qualitative observations showed the following.

The number of population peaks varied from 1–3 per year. The first peak occurred in the third week of April (year 1981), third week of May (year 1980) or the first to second week of June (years 1979 and 1982). Usually it occurred during the pre-monsoon season (figure 1). There was no apparent temporal correlation between the subsequent peaks and the rainfall. In 1980 and 1982 the first peak itself was large, causing total defoliation in most plots, but in 1979 and 1981 the first build-up was smaller.

A second peak occurred in 3 of the 4 years. In 1979 and 1981 it was high in most plots, causing near total defoliation, but in 1982 it was very small and was noticed only in the two plots located in Block I. It was also small in one of the plots in Block II in 1981. The

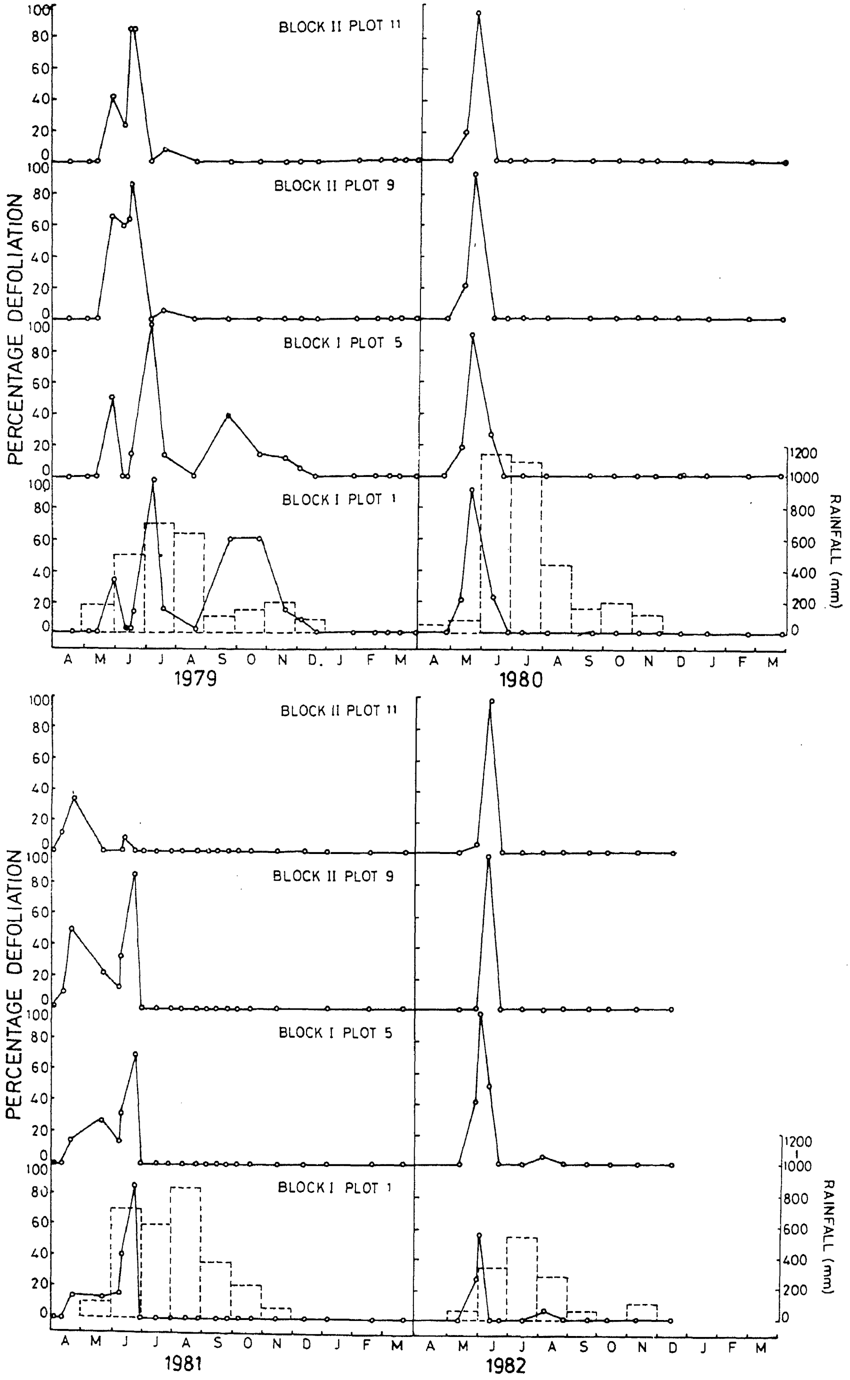

Figure 1. Seasonal incidence of defoliation caused by *H. puera* in the 4 study plots at Nilambur, over the 4-year period. The bars show monthly rainfall.

interval between the first and second peaks ranged from 18–60 days. In the same year (1979) it was 30 days in Block I, but only 18 days in Block II. In 1981 and 1982 it was 60 days in most plots. The life cycle of *H. puera* is usually completed in 18–27 days (Beeson 1941; unpublished observations from our laboratory) and the varying intervals between the two population peaks suggest that the second peak does not necessarily represent the second generation of the insect. This is particularly evident from the data for 1979, when the first peak occurred on the same date in both the blocks, but the second peak occurred after 18 days in Block II and after 30 days in Block I.

A third peak was noticeable only in 1979. In Block II it was very small and occurred about 30 days after the second peak. That it represented a new generation of larvae was confirmed by field notes. In Block I, the third peak was higher and occurred about 75 days after the second peak. In both the plots, this peak was flatter than earlier peaks, suggesting that the insect population had a mixed age-structure, representing larvae of more than one overlapping generations.

In general, while plots within the same block showed similar trends, there was greater difference in the time of occurrence of infestation between the two blocks situated 4 km apart. Some defoliation peaks not represented in the study plots were observed in other plantations adjacent to these plots. One such peak occurred in early August 1980, when all trees in about half a hectare were totally defoliated.

3.2 *Infestation characteristics*

Detailed observations on infestation characteristics started on 14 April 1984 when a large population of approximately 3-days-old larvae was noticed on some trees near the house, for the first time in the year. In the immediate surroundings of the house, the infestation was confined to 8–10 of about 30 trees present. Trees with comparatively tender leaves were the most heavily infested and the larvae were distributed throughout the canopy with greater concentration on the most tender, terminal leaves. Trees with comparatively older leaves had a smaller larval population, mostly confined to the topmost and peripheral layer of the canopy consisting of tender leaves. The infestation was spread over a vaguely delimited patch, and similar trees in adjacent areas were unaffected. In other areas within the KFRI Campus, similar or larger patches of infestation were found at the same time, but some areas were free of infestation. These larval populations differed in age only by a day or two.

A day after sighting the infestation, small larvae were seen hanging on silken threads and many of them landed on lower leaves and within two days, some trees with tender foliage were completely stripped of the leaf blades, leaving only the petioles and larger veins. The larvae then moved to adjacent trees with older foliage while a few descended to the ground and fed on saplings. Where the initial population was small and confined to tender leaves at top, the larvae, after consuming the tender leaves, descended to older leaves of the same tree and continued feeding. In many trees, all the older leaves were eaten up by larvae which migrated either from the upper canopy or from adjacent trees (figure 2C). Usually the population of larvae on infested trees was very high, with each tender leaf harbouring 50–100 larvae (figure 2A) and the sound of faecal pellets falling continuously on the lower leaves and on dry leaves on ground could be heard very clearly. Feeding continued throughout the night. After 5 days the nearly mature larvae descended to the ground and wandered all over, while some continued to feed on the

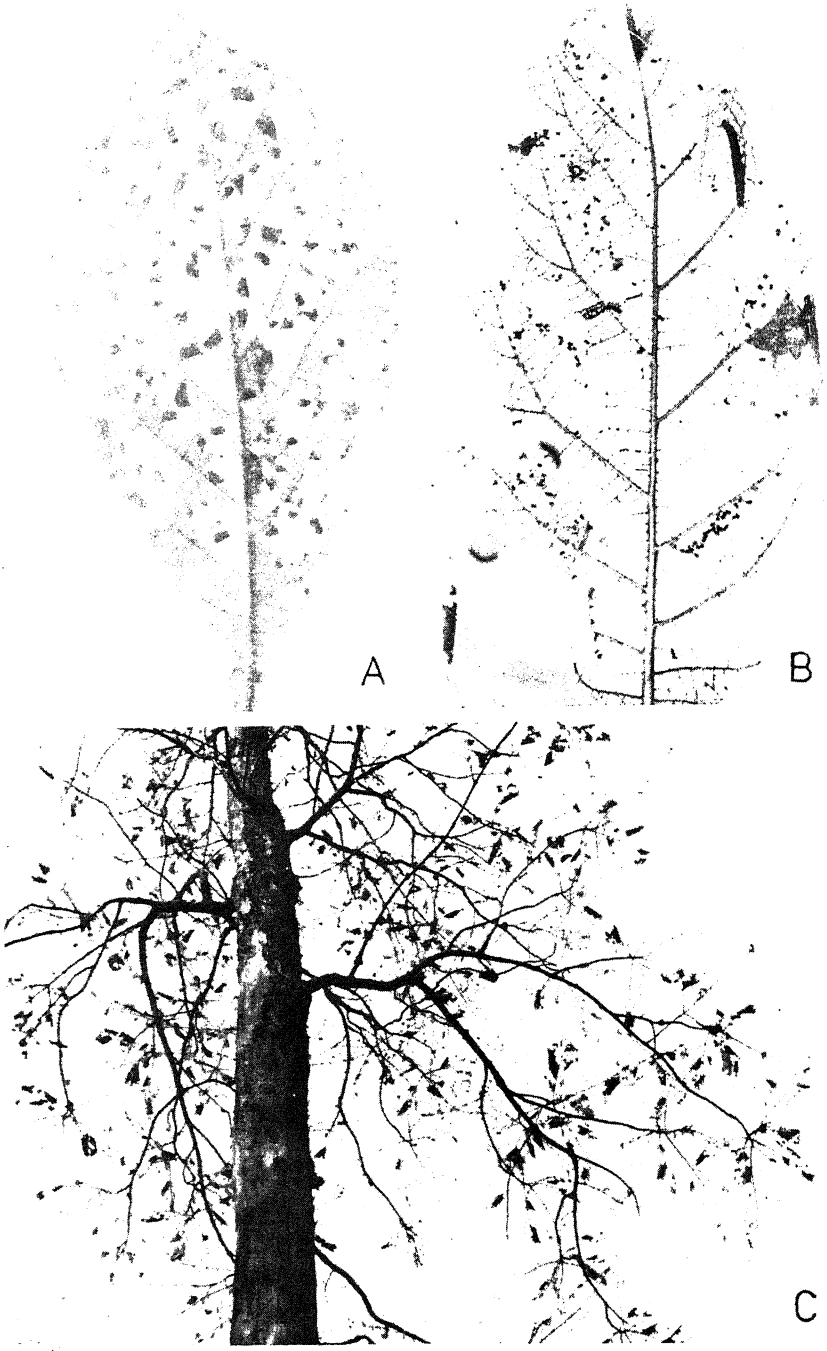

Figure 2. Infestation characteristics of *H. puera*. **A.** A heavily infested tender leaf showing about 80 young larvae harboured within small leaf folds. **B.** Late larvae feeding on mature leaf of a sapling. **C.** Bottom portion of a tree showing infestation of mature foliage by larvae which migrated from the top canopy.

leaves of saplings (figure 2B). Some larvae also nibbled on lantana leaves. Thus, many trees were completely stripped during about 8 days of larval period. On 19 April, i.e., 5 days after the observations began, large groups of full-grown larvae were seen crawling all over the ground and on the walls of the house. A small number still continued to feed on the trees and moved to the ground over the next two days. Three days after the mass movement of the mature larvae, pupae were noticed for the first time, on the ground, under dry leaves. Some pupae were also found on the bare walls and floor of the house. Feeding larvae were not noticed any more.

On 25 April, i.e., 3 days after the first pupae were formed, moths began appearing at light. Large numbers were attracted to domestic lights in the following days. A few moths were also seen during early mornings, resting on the leaves of teak saplings and sometimes on other plants. The moth population reached a peak on 28 April and declined thereafter, reaching very low levels on 1st and 2nd May and nil on 3rd. From these observations, the period from egg laying to peak moth emergence was estimated to be about 20 days (from 8–28 April).

Since a large population of moths was present in the observation area, a much bigger second infestation was anticipated. However, no significant infestation occurred, in spite of the presence, in the same area, of trees possessing tender leaves (including those partially refoliated after the insect attack). Nowhere in the campus was a significant infestation noticed. However, a few larvae were seen on saplings, first on 29 April. On subsequent days, a few eggs and a few larvae of uneven age were seen, again only on saplings. Nearly mature larvae were seen on 8 and 11 May, alongwith smaller larvae. Small larvae were again noticed on 23 May. Older trees were not infested, although a few larvae were occasionally found on lower branches. These observations showed that following the heavy infestation, only a small, uneven-aged population existed in the same area and it was confined to the saplings. After May, no larva was seen until late September when a small number appeared, again on saplings. Small populations with overlapping generations were also found in October, November and January, causing visible, but negligible defoliation of saplings. Although a few larvae remained on the lower branches of trees, none were seen in the top canopy.

3.3 *Spatial distribution of infestation*

Figure 3 shows the survey route and the major locations where old or new infestations were noticed between 15 and 22 May 1984. In southern Kerala, a distance of over 30 km from Kulathupuzha to Konni, via Achencoil, was covered through teak plantations and it was found that extensive defoliation had already taken place by 15 May. Earlier field notes revealed that two waves of defoliation had occurred, the first around 10 April, in small patches, and the second around 23 April, over more extensive areas. On 15 May, new flushes of leaves were present in most areas. A few moths and a few late larvae were seen, but there was no visible damage. Longer distances were covered north of Trichur, of which about 135 km along the road had bordering teak plantations. Of this, about 27 km in total had plantations which had already suffered defoliation. The new flushes indicated that infestation had occurred 2 or 3 weeks earlier. An extent of about 30 km, distributed over different areas, had plantations with currently active infestation. The larvae were in different stages of maturity at different observation sites, but within a continuous patch of infestation the population was even-aged. Some typical examples

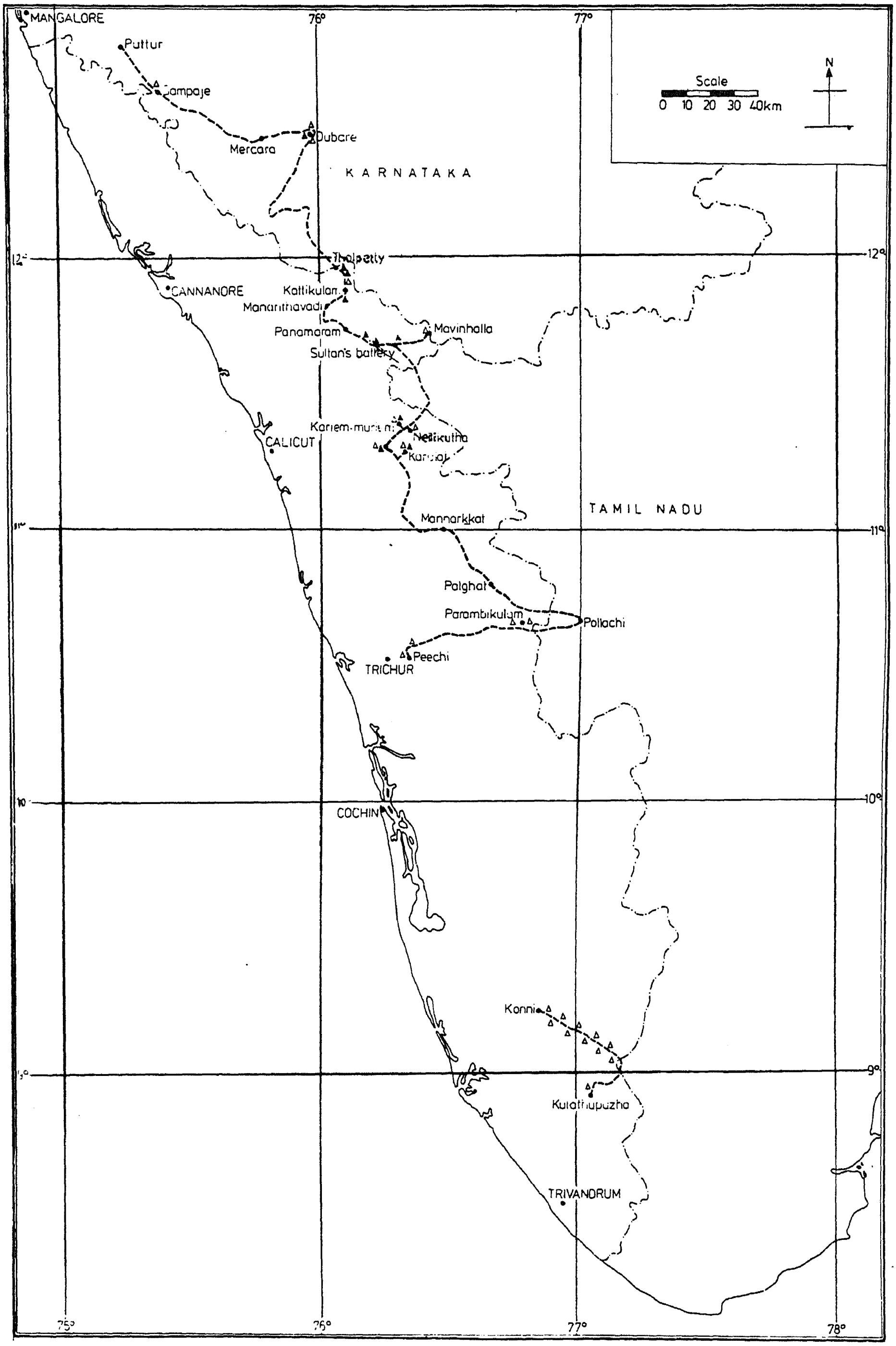

Figure 3. Map of Kerala showing the survey route and the major localities where defoliation was observed. Along the route, old defoliation is indicated by open triangles and current defoliation by closed triangles.

of the distribution of old and new infestations are shown in figure 4. It is evident that infestation occurred in discontinuous patches. Patches of current infestation were separated from patches of old infestation by a few kilometers.

At Nilambur, reinfestation of the same plantation was found in some places, viz., Kariem-Muriem, Karulai and Aravallikkavu. At Kariem-Muriem, very young larvae were present on 21 May on trees which had begun to reflush after a previous defoliation in the last week of April. Earlier records showed that there was yet another previous infestation in the first to second week of April, confined to some patches of this area. At Aravallikkavu, a large population of small larvae was present on trees which had been previously defoliated and which had by then developed a new pair of leaves just reaching one quarter of the full size. It was evident that these larvae would not be able to complete development on the available foliage. The quick succession of a new generation of larvae indicated that they did not represent the progeny of the previous generation in the same plantation.

In addition to plantations, over a distance of about 100 km along the route there were isolated teak trees, forming a more or less continuous chain, particularly between

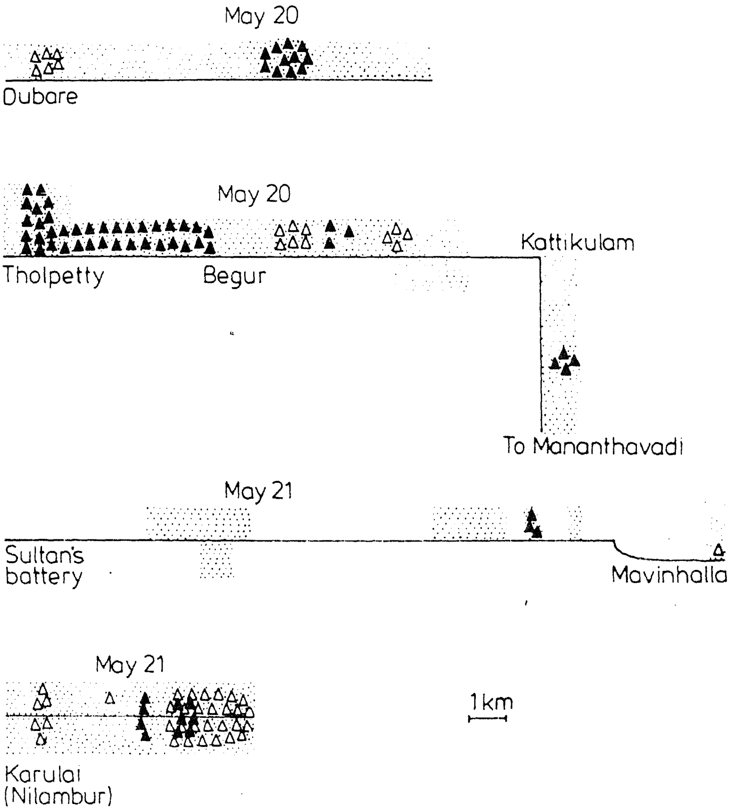

Figure 4. Spatial distribution of *H. puera* infestation in selected roadside teak plantations. The plantations are shown by stipling, patches of old infestation by open triangles and patches of current infestation by closed triangles.

Nilambur and Parambikulam. These trees were not attacked, except at one place abo 20 km north of Sultan's Battery.

In a natural forest near Mavinhalla, where teak was prevalent, most trees were free infestation. But at one location, about 10 trees within a radius of about 50 m we affected. A few trees in the centre showed heavy defoliation but in the surrounding tre most damage was confined to the top. About 50 m away from this patch, another tr was attacked, but only the top leaves were damaged, and the surrounding trees were fr of infestation. A larger patch of defoliation within a natural forest stand was al noticed at Anakkad, near Kushalnagar in Karnataka.

3.4 *Miscellaneous observations*

On some occasions during May–June, concentration of young larvae on the top cano of trees, as noticed at Peechi, was also seen in other areas.

Perusal of the field notes over several years revealed that small numbers of acti larvae have been observed at several places during the 'off-season'. These included, oth than those already noted, records of larvae in October (12–19 October 1978 and October 1980 at Nilambur as well as Peechi), November (5 November 1980 Nilambur; 24 November 1982 at Thenmala; 11 November 1983 at Peechi; 16 Novemb 1984 at Konni), December (13 December 1976 at Nilambur; 11 December 1980 Peechi; 14 December 1982 at Nilambur) and March (15 March 1984 at Kulathupuzh Of these, the one in November 1983 at Thenmala was a fairly heavy incidence confin to a group of nursery beds and adjacent 2–3 year-old saplings. The one in March 1984 Kulathupuzha was a typical patchy infestation on tree tops. All others were sightings a few larvae on young plantations.

4. Discussion

Several characteristics of infestation revealed by this study indicate migration of t moths. These include (i) the sudden, heavy infestation of trees, (ii) concentration of e laying on the top canopy of trees to the exclusion of saplings at ground level during t early part of the year, (iii) failure of the large population of newly emerged moths give rise to a significant new generation of larvae in the same locality, (iv) t distribution of infestation in discrete patches, well separated in space during the ea build-up phase, and (v) the varying intervals between successive defoliation peal which show that the peaks represent, not the generation peaks, but waves of mo immigration (except during the later part of the year when overlapping generatio occurred, as in 1979). Such overwhelming evidence in favour of migration has not be reported earlier, although the recent reports of Vaishampayan and Bahadur (1983) a Vaishampayan *et al* (1984) suggested the possibility of migration.

The migration of moths appears to begin in April–May and end by June–July in mc years, i.e., it is mostly confined to the 2–3 months period preceding the onset of hea monsoon rains. In some years yet another wave of migration seems to have occurred August–September since sudden defoliation without continuous previous generatio was noticed. For example, at Nilambur such a wave of defoliation occurred in 1979 Block I, in 1980 in an area outside the study plots and in 1982, again in Block I (thou the peak was very small). Beeson (1928) also reported the occurrence of a wave of heavy defoliation in September–October at Nilambur. There is no evidence of migration after this period. The flat peaks of defoliation in October and November 1979 in the Nilambur study plots (figure 1) shows the existence of a resident population of the insect representing overlapping generations. The sightings of larvae, albeit in small numbers, in October, November, December, January and March at Peechi and other places also indicate the presence of a resident population of the insect following the migratory phase. This is not reflected in the defoliation curves for most years, apparently because the populations were too small to cause measurable defoliation. A residual, non-migratory population also existed during the period in which migration occurred, as clearly shown by the observations at Peechi. Such a residual population was also seen at Konni following heavy defoliation. They confined their feeding chiefly to the understorey.

Based on absolute absence of moths in the light trap for a period of 6 months from January–June at Jabalpur, Vaishampayan and Bahadur (1983) concluded that *H. puera* does not breed in the same area on any host plant during this season, which necessitated postulation of either migration to some other areas for off-season breeding or occurrence of diapause in the pupal or adult stage. Our observations show that the insect is active throughout the year although the population may be very low and undetectable when the search is confined to a small area. In the absence of other information, a study of figure 1 would have also led to a similar conclusion, that the insect is totally absent for a long period from July to next April–May in most years and at least for a period of about 4 months from December–March in all years. But pooling the observations for all places and all years, it may be seen that the insect was present throughout the year, except in February. The rarity of sightings during December–March, the normal leafless period of teak, is understandable since most observations were made in plantations. Although teak is deciduous, observations have shown that in natural forest there is enough phenological variation among individual trees within an area and among populations of trees located in different areas within a larger geographical region that tender leaves are available continuously, though not at the same place, to sustain a residual population of larvae. *H. puera* is also known to survive on many alternate hosts (Beeson 1941). In the light trap study of Vaishampayan and Bahadur (1983) the trap was located about 20–30 km away from the teak forest. The possibility of a residual population not being picked up due to the long distance involved and the lack of responsiveness of the moths at this season (the moths may travel such long distances only during the migratory phase) cannot be ruled out.

The occurrence of a residual population during the off-season can be taken as evidence against diapause, although it is theoretically possible to argue that the entire population need not undergo diapause. But as will be shown later, the comparative scarcity of the insect during the 'off-season' can be explained otherwise, without postulating diapause. Other evidences against diapause are the temporal and spatial discontinuity of the first major infestation. If the insects have been in diapause, within a locality, they should become active at about the same time and be distributed more uniformly. These considerations clearly rule out diapause.

While the occurrence of migration is a certainty, the details remain to be worked out. All major defoliations in India have been noticed between April and September and there is no record of large-scale off-season breeding anywhere, although this was suspected by Vaishampayan and Bahadur (1983). If the existence of a residual population during the off-season as suggested by the present study is accepted, it is no longer necessary to look for places where large-scale off-season breeding may occur. In the light of our present understanding, we propose a tentative model of the population dynamics of *H. puera* (figure 5). According to this model, during the off-season, a residual, active population of the insect exists on host trees which possess tender foliage suitable for oviposition and survival of the early larval instars. Such a population is more likely to occur in natural forests because of continuous availability of tender foliage due to natural phenological variation of teak, and occurrence of alternate hosts. With the onset of general flushing during February–March, the population starts increasing, generation by generation, as more and more tender foliage becomes available. When the population density reaches a critical level, the migratory behaviour is triggered. Larval crowding leading to exodus movement of full grown larvae to pupation sites probably plays an important role in initiating the migratory behaviour. The subsequent steps, which lead to a large increase in the population are shown in figure 5. The population build-up may proceed in the general direction of early flushing to late flushing areas. A south to north trend in the progress of infestation is indicated by our limited spatial distribution study. Heavy defoliation of small groups of isolated teak trees inside natural forests have been noticed prior to occurrence of large-scale defoliation in plantations. Such early build-ups which may start in February–March with the beginning of general flushing may usually escape attention. What usually catches the eye are comparatively larger infestations in plantations, beginning in April–May. Vaishampayan and Bahadur (1983) were able to trap large numbers of moths at least 20–30 km away from teak forests, which suggests that the moths are able to migrate at least 20 km, and probably more. Recent studies on the armyworm moth, *Spodoptera exempta* in Kenya using radar and infra-red optical techniques (Riley *et al* 1983) indicated that moths climbed to altitudes of several hundreds of meters above ground level and covered at least 20 km downwind during the first half of the night. Studies by Rose *et al* (1983) on the same species indicated that the moths could migrate at least 140 km downwind, probably in one night. *H. puera* also probably has the potential to migrate long distances. But based on our observations of the distribution of attack which indicated a minimum distance of 4–5 km between old and new infestations during the build-up phase, we postulate that the moths normally migrate short distances of 5–10 km when suitable host trees are available within this distance. There is not enough evidence to support this hypothesis because even long-distance migration in small groups can lead to the observed distribution pattern. But normally, in long-distance migration, one may expect bigger swarms which may infest a larger area. It appears that the moths are capable of migrating long distances, but the flight is cut short when plantations with tender foliage are available, probably because of the arrestant effect of a characteristic olfactory profile of newly flushed teak plantations. However, a minimum distance of 4–5 km may be covered before they settle.

The geometric increase of the insect population as a result of continuous generations which exploit newer and newer territory by gypsy-like migrations, is curtailed by several factors. During June–July, the heavy monsoon, with spells of continuous rainfall may adversely affect the survival of the insects either directly or indirectly. The most important limiting factors, however, are (i) leaf maturity, (ii) natural enemies, and (iii) density-dependent food depletion. The moths lay eggs only on tender leaves and apparently, the early instars are able to survive only on them. In Kerala, most trees have

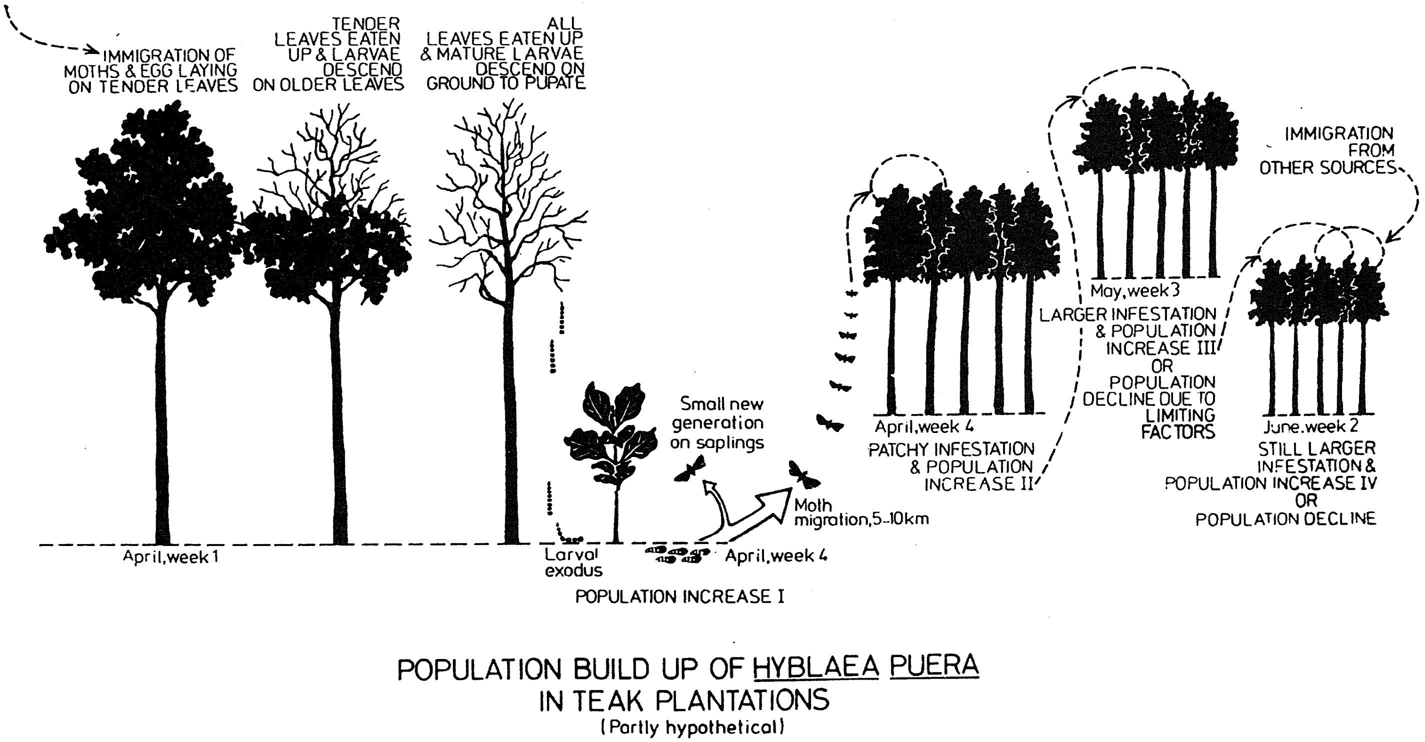
IMMIGRATION OF MOTHS & EGG LAYING ON TENDER LEAVES
TENDER LEAVES EATEN UP & LARVAE DESCEND ON OLDER LEAVES
ALL LEAVES EATEN UP & MATURE LARVAE DESCEND ON GROUND TO PUPATE
April, week 1
Larval exodus
Small new generation on saplings
Moth migration, 5-10km
April, week 4
POPULATION INCREASE I
April, week 4
PATCHY INFESTATION & POPULATION INCREASE II
May, week 3
LARGER INFESTATION & POPULATION INCREASE III OR POPULATION DECLINE DUE TO LIMITING FACTORS
IMMIGRATION FROM OTHER SOURCES
June. week 2
STILL LARGER INFESTATION & POPULATION INCREASE IV OR POPULATION DECLINE
POPULATION BUILD UP OF HYBLAEA PUERA IN TEAK PLANTATIONS
(Partly hypothetical)

Figure 5. Schematic diagram showing the proposed model of population dynamics of *H. puera* in teak plantations.

fully mature leaves by July and they are no longer suitable for sustaining a large population of larvae, particularly the early instars. A number of predators and parasites of *H. puera* larvae are known (Chatterjee and Misra 1974) and some of them have been found very effective; for example, on one occasion, a large epidemic of larvae at Pullamkandam near Peechi was abruptly cut short by parasites. When examined on 9 June 1977, almost every larva, mostly middle-aged, was dead or moribund and the few active ones had fresh eggs of a tachinid parasite on the body surface. A batch of 200 larvae collected and kept in small groups with food, yielded only one adult moth and a dead pupa; the remaining larvae yielded unidentified hymenopteran parasites and a tachinid parasite which was later identified as *Palexorista solennis*. Recent investigations (Sudheendrakumar V V, unpublished results) have revealed that the early generations are comparatively free of parasites but later generations suffer heavy mortality due to hymenopteran and tachinid parasites. Dead larvae showing typical symptoms of bacterial disease have also been found occasionally and in the laboratory, bacterial disease is a major problem in rearing the insect. Viral diseases are also suspected in the field. During epidemics heavy predation by birds also contribute to mortality. The third major limiting factor, density-dependent food depletion, operates when a previously defoliated plantation is re-infested within a short period. Examples of this situation may be more prevalent in large contiguous plantations, as observed at Aravallikkavu and other places at Nilambur. Due to the combined action of these mortality factors, the population crashes down and returns to the residual level, usually by July, and sometimes after another peak in August–September, in Kerala. This cycle of ups and downs is repeated every year.

The gypsy-like, short-range migration confers two major advantages to the insect. (i) Movement facilitates discovery and utilization of areas with new flush of leaves. The sites of earlier infestation can be utilized again after the trees have put forth a new flush. (ii) Migration is a method of escape from larval parasitism since the progeny of the early generations moves away from the parasite population built up during their development. But with larger spread of the population, the parasites also catch up. The parasites appear to be the most dominant limiting factor in the population dynamics of *H. puera*.

As is obvious, some steps of the above model of population dynamics need confirmation. If the existence of a residual population during the off-season and step by step build-up of the population with short-range migration are confirmed by further study, simple methods can be employed to control the epidemics of this insect in plantations. In large plantations, it is possible to locate sites of initial build-up by surveilance during critical periods. Control operations can be carried out in the affected patches to prevent emergence of the moth population and its subsequent spread to other areas. Application of a biocide or chemical insecticide over the ground on which the mature larvae descend to pupate may prove useful. This will be much simpler, economical and environmentally less hazardous than aerial application of insecticides that has been tested in the past (Singh 1980) against *H. puera*.

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Functional morphology, anatomy and histology of the digestive organs of the catfish *Plotosus canius* Hamilton*

M SINHA
Central Inland Fisheries Research Institute, Barrackpore 743 101, India
Present address: Central Inland Fisheries Research Centre, PO: Netaji Subhash Sanatorium, Distt. Nadia (W.B.) 741 251

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Abstract. The morphology, anatomy and histology of the organs concerned with capture and digestion of food of the canine catfish-eel, *Plotosus canius* Hamilton, have been described. It shows a primitive organisation of digestive organs in the presence of only one type of secretory cell in the gastric glands and diffused pancreatic cells, embedded within the intrahepatic cells. The study indicates the species to be a carnivore with characters of both primitive, as well as, developed carnivorous nature. While discussing, the structural peculiarities observed, have been correlated with type of food generally encountered in the gut of the species.

Keywords. *Plotosus canius*; morpho-histology; digestive organs.

1. Introduction

Examination of gut-contents is the only possible method to infer about the feeding habit of aquatic animals, like fish, in whose case it is not easy to directly observe them feeding in their natural habitats. Pillay (1952) observed that gut-contents may sometimes contain only emergency food items and thus, any conclusion drawn on its basis, as regard to the natural food of the fish, may be, at times, totally erroneous. He, thus, suggested the morpho-histological study of the organs concerned with capture and digestion of food, in addition to the gut-contents, to overcome this limitation and to infer conclusively about the feeding habit of any particular fish species.

Thus, while studying the food and feeding habit of the canine catfish-eel, *Plotosus canius* Hamilton, it was felt essential to study the structure and function of the alimentary canal and its associated structures and to correlate the same with the type of food encountered in the gut (Sinha 1984).

2. Material and method

Live specimens of *P. canius*, brought to fish assembly centre at Kakdwip (Hooghly-Matlah estuary), were used for this study. For morphological and anatomical studies, the fishes were dissected along the ventral side of the abdomen. Detailed morphological studies of the buccopharynx, alimentary canal, liver and gall bladder were made by examining the structures by means of a hand lens. The dispositions of the mucosal folds of the different regions of the alimentary canal were studied under a binocular microscope by administering a longitudinal incision on each region, initially, and mounting the tissue in glycerine subsequently.

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For histological studies, small pieces from different regions of the alimentary canal were removed from live fishes, washed in normal saline and fixed in aqueous Bouin's fluid for 12–24 hr in separate tubes. The liver was removed and fixed separately. Transverse paraffin wax sections (5–8 μ thick) were then made, stained in Delafield's Haemotoxylin, counter stained by Eosin and mounted in DPX, following standard methods. The histological structures of different organs were studied from these sections.

For total length–gut length ratio, the total length of the fish and the length of the gut (oesophagus to rectum) was recorded of 239 fish, ranging in length from 40 to 538 mm, collected for other biological studies.

3. Observations

3.1 *Morphology and anatomy*

The adaptations of the alimentary canal of fishes to their food habits are particularly evident, amongst others, in relative length of the gut. This ratio, calculated in case of *P. canius* individually, showed no marked difference with the increase in the size of the fish. It did not show any seasonal variation too. Figure 1 depicts the observed gut lengths of fishes in relation to their total lengths. The ratio was found to range from 0·78–1·19, with an average of 1·08 and with a coefficient of variation of 7·46% which is not significant at 238 d.f.

The alimentary tract of *P. canius* is not a uniform tube throughout, but is rather thrown into a number of turns. Except for the mouth and buccopharynx, the other regions are partially differentiated morphologically but their differentiation is clearly marked anatomically because of difference in the nature of their mucosal folds. The regions which can be recognised in the alimentary tract of this species are: mouth, buccopharynx, oesophagus, stomach, intestine and rectum. The details of the morphological, anatomical structures of these as well as the associated structures, are as follows.

3.1.1 *Mouth:* The mouth of *P. canius* is comparatively small. The gape of the mouth is transverse. The upper jaw is slightly longer than the lower, so that mouth opening is distinctly downwards (figure 2). Upper lip is thicker and cartilaginous.

3.1.2 *Buccopharynx:* The buccal cavity is narrow anteriorly and wider posteriorly, whereas, it is *vice versa* in the pharyngeal cavity (figure 3). The base of the cranium forms the roof of the buccopharynx, whereas, its side and floor are supported by branchial arches and median urohyal respectively. The mucosal thickening at the anterior part of the floor is suggestive of a tongue-like structure. The roof of the buccopharynx contains maxillary, vomerine and pharyngeal teeth, whereas, the floor is provided with mandibular and horny pad teeth. The maxillary teeth are small, conical in shape and arranged in three rows. They remain outside the buccal cavity when mouth is closed (figure 2). The vomerine teeth are molar-like, three to four rows in the middle and one or two rows on sides. The pharyngeal teeth are small, conical in shape

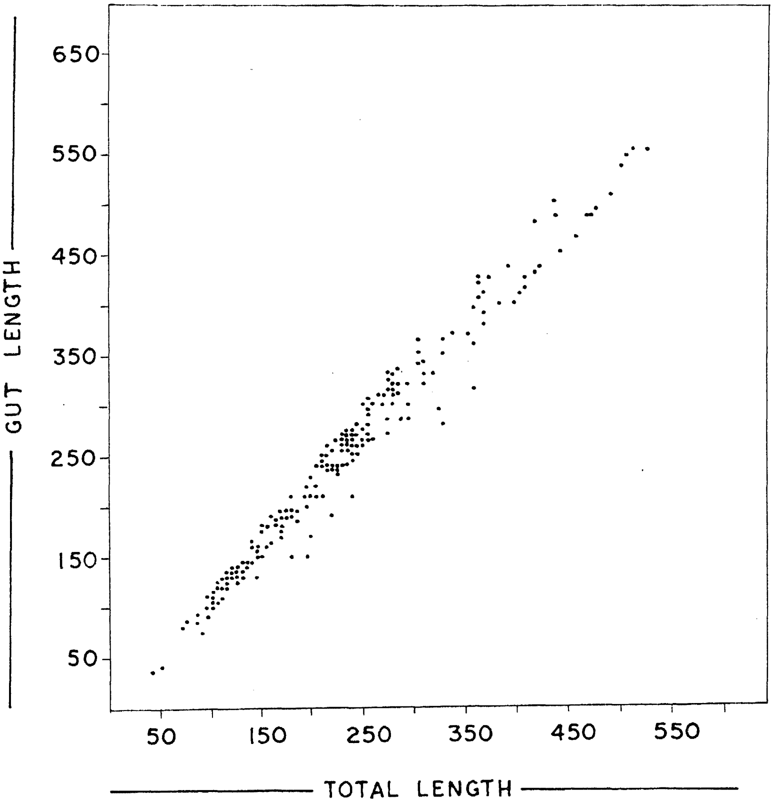


Figure 1. Gut length and total length relationship of *P. canius*.

and are placed in two semi-circular patches, supported by the pharyngo-branchials of the second and third gill-arches. Mandibular teeth are borne on dentaries and are like vomerine teeth in shape. They are the largest amongst all buccopharyngeal teeth. The horny pad teeth are lodged on a pair of horny pads and are nearly as large as the pharyngeal teeth. The horny pads are fused with the inner surface of the fifth branchial arch which is reduced to a single strong bone on either side and is without any gill-filament, resembling 'os pharyngeous inferior' of Goodrich (Al-Hussaini 1949).

It is evident from the above that dentition in *P. canius* is heterodont. All teeth in the buccopharynx point backwards. They are slightly inclined in such a manner that vomerine and pharyngeal teeth of the upper side face mandibular and horny pad teeth of the lower side of the buccopharynx respectively.

The gill-rakers are short, being the longest on the first branchial arch and gradually reduced thereafter. Their number is 21, 18, 15, 9 and 7 on first, second, third, fourth and fifth branchial arches respectively. In the case of third, fourth and fifth branchial arches they give an appearance of conical shaped teeth, set wide apart.

3.1.3 *Oesophagus:* Buccopharynx leads into oesophagus which is tubular, of a greater calibre in front, passes dorsal to the pericardial chamber and after getting through the septum transversum enters into visceral cavity (figure 5a). In the visceral

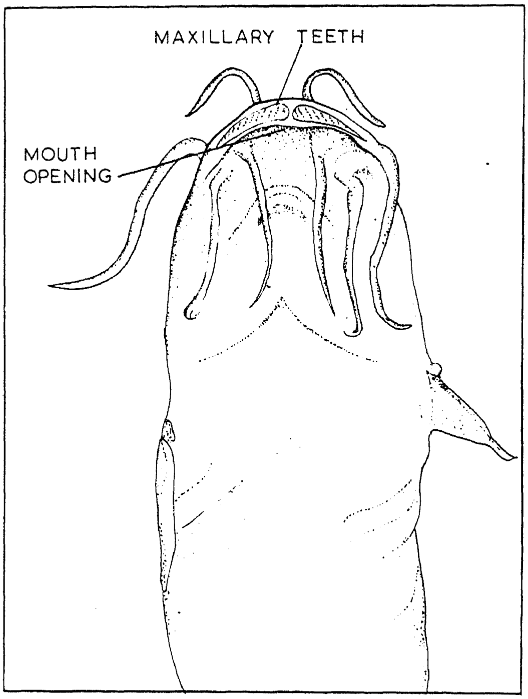


Figure 2. Ventral view of the anterior portion of *P. canius*.

cavity it passes over the lobes of the liver. There is a slight external indication of the passage of oesophagus into the stomach, but if the oesophageal tube is cut open, the line of demarcation is well marked because of difference in the nature of mucosal folds. The mucosa of the oesophagus is of thick reticular longitudinal folds provided with thin transverse folds in between (figure 5b).

3.1.4 *Stomach:* *P. canius* lacks a true sac-like stomach, commonly found in carnivores. The stomach can only be differentiated from the rest of the alimentary canal for its being a little wider from the rest (figure 5a) and because of specific structures of the internal mucosal folds. The pyloric region of the stomach is small with a slight external constriction at its junction with the cardiac stomach. The mucosa of the cardiac stomach is provided with thick longitudinal folds, with a few transverse folds in between connecting them (figure 5c).

3.1.5 *Intestine:* The intestine takes its origin from the pyloric end of the stomach (figure 5a). It is convoluted and is held in position by the mesentery. The bile duct enters the intestine close to its origin from stomach. Variations in diameter and dispositions of the mucosal folds occur in the anterior and posterior portions of the intestine. The anterior part of the intestine has longitudinal mucosal folds, set wide apart, with reticular thin mucosal folds in between (figure 5d). The same in the posterior part is much closer with no reticular connections (figure 5e).

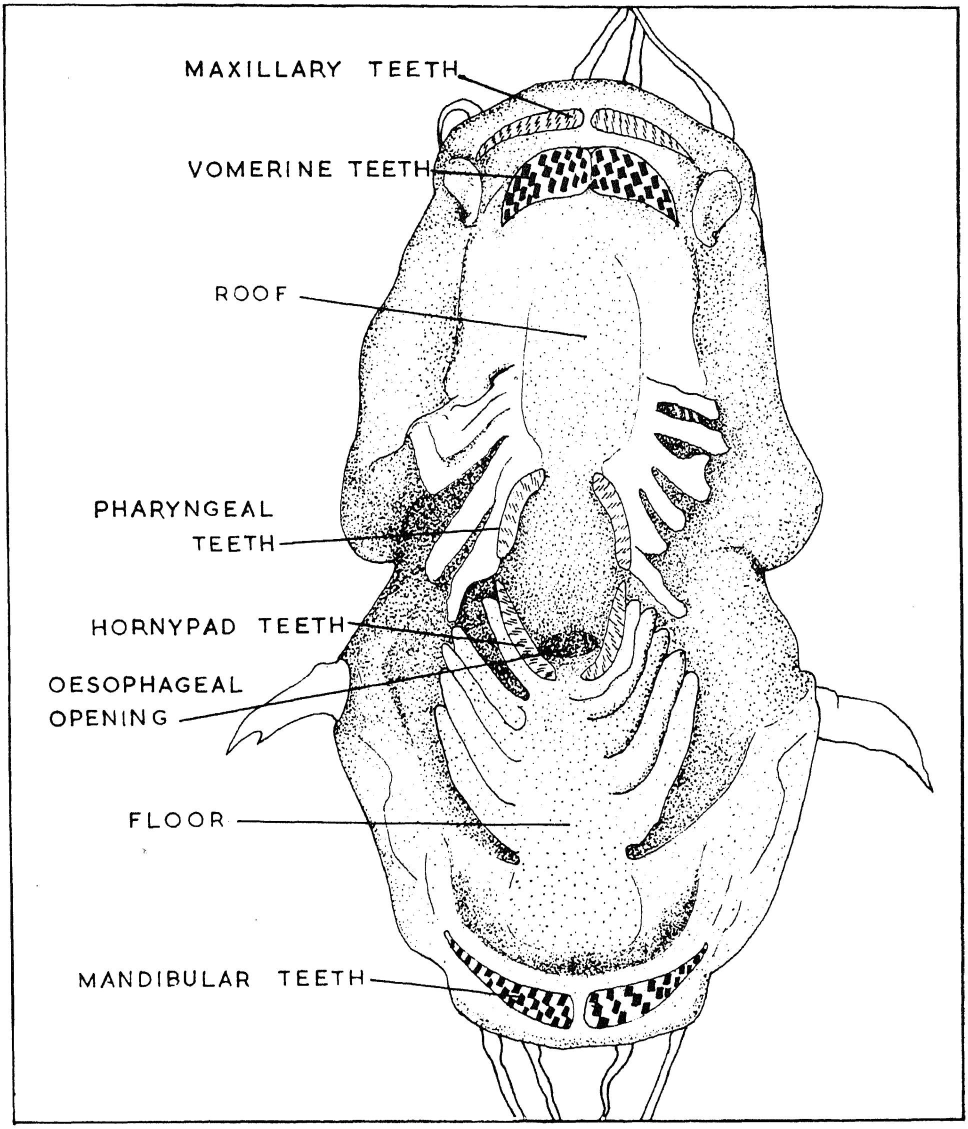


Figure 3. Bucco-pharyngeal region of *P. canius*.

3.1.6 *Rectum:* The rectum is short and cannot be distinguished externally from the intestine proper (figure 5a). The mucosal folds here are in the form of thick longitudinal bands (figure 5f).

3.1.7 *Associated structures: Liver*: The liver is the principal digestive gland in *P. canius*. It is reddish brown in colour, convex on its outer surface and surrounds part of the alimentary canal on its ventral surface. The gland is not divided into distinct lobes.

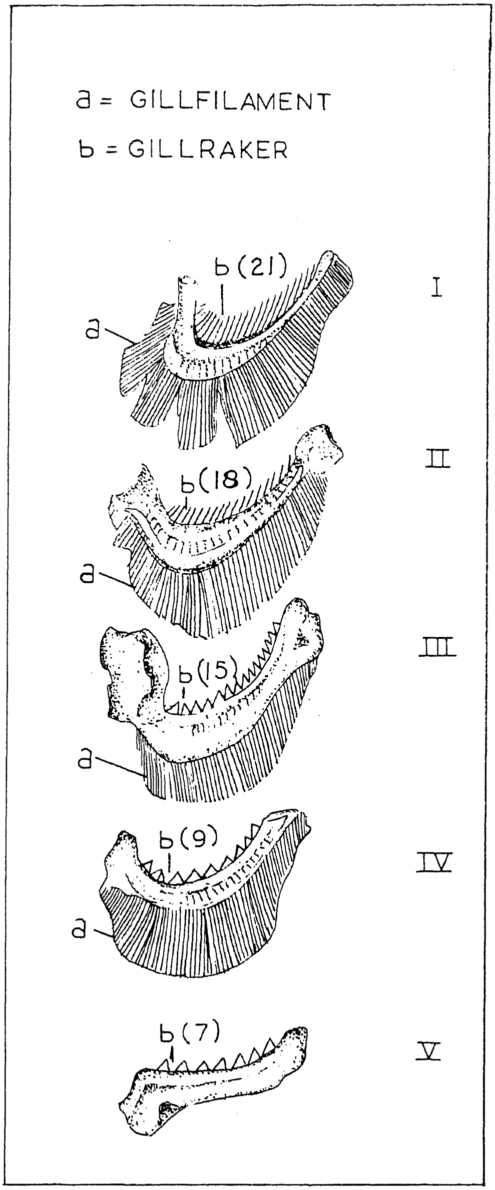


Figure 4. The five branchial arches of *P. canius.*

Gall Bladder: The gall bladder is a small, round, greenish, sac-like structure lying below the right of the liver. The bile duct is short and opens into intestine, close to its origin from stomach.

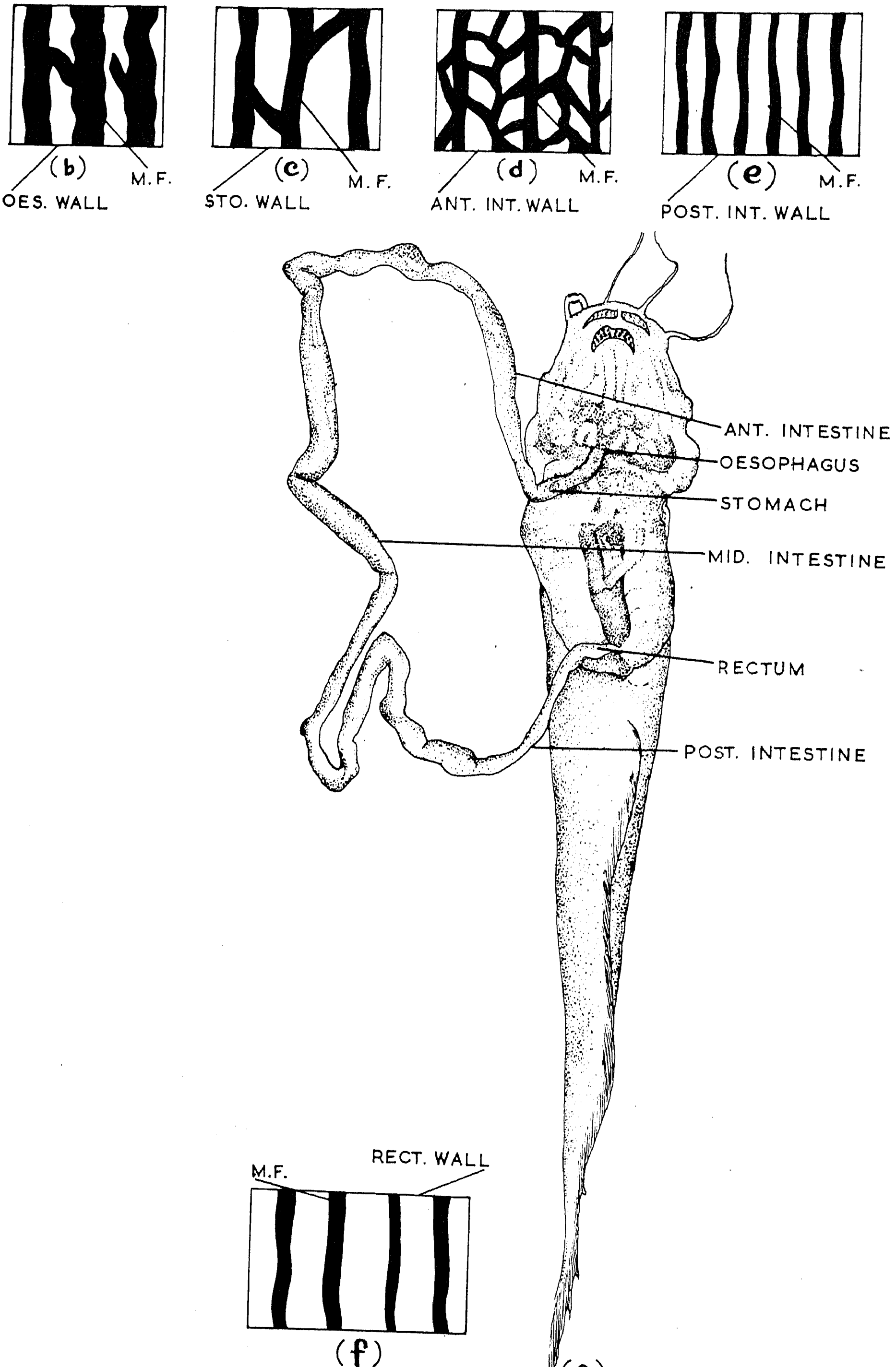


Figure 5. Alimentary system of *P. canius*. (a) Nature and disposition of alimentary tract; (b)–(f) mucosal folds of different regions.

3.2 *Histology*

The alimentary canal of *P. canius* is made up of several layers which differ in various regions of the tract. The histological details of the alimentary canal are as follows:

3.2.1 *Pharynx* (figures 6a, b): The wall of the pharynx is made up of three layers, viz. mucosa, submucosa and muscularis mucosa. The mucosa is composed of stratified epithelium and gets folded to form villi-like projections. The epithelium is composed of

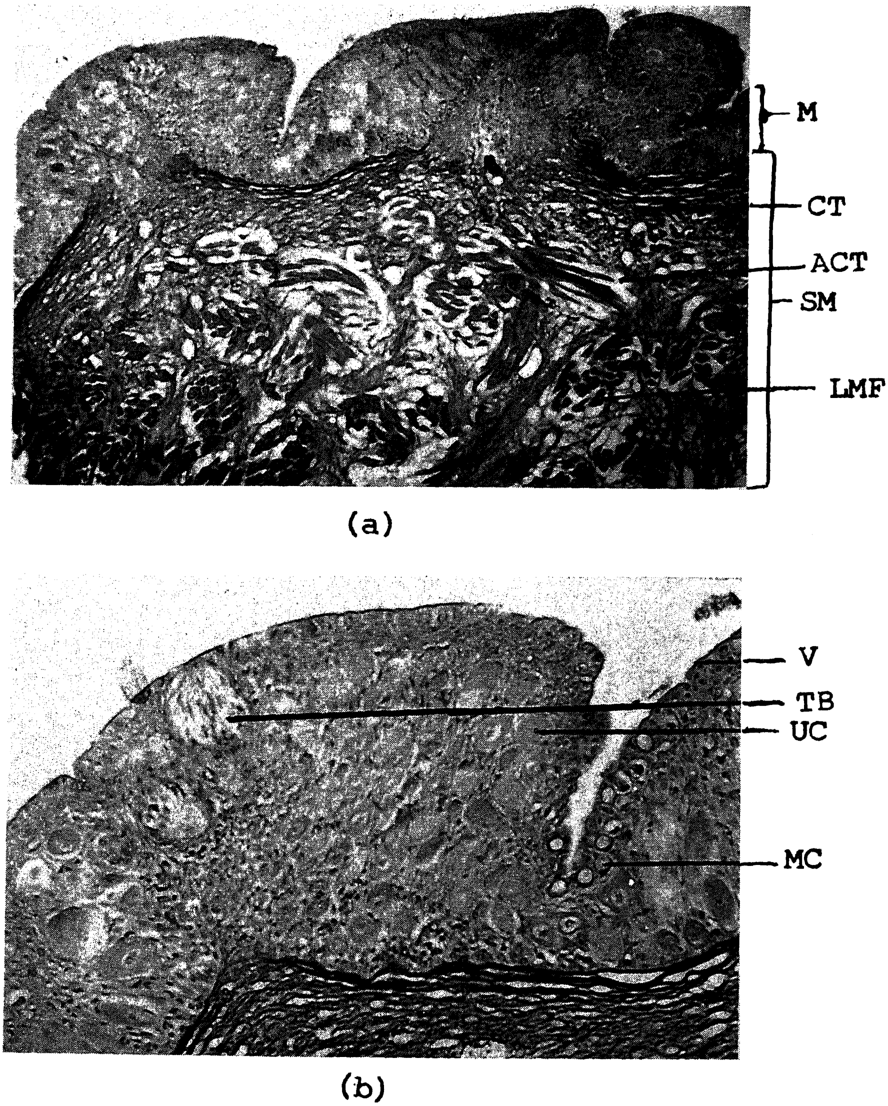


Figure 6. T.S. of pharynx of *P. canius*. (a) × 40; (b) × 100.

mucous cells, taste buds and undifferentiated cells. The mucous cells are generally arranged superficially and are located on the side walls of the crypts. They are either elongated or oval and are arranged with their long axis perpendicular to the surface of the epithelium. Those that open to the exterior are goblet shaped. The mucous cells are derived from the undifferentiated cells and as such their different stages could be made out throughout the thickness of the epithelium. Taste buds are a few in region but are present both in anterior and posterior part of the pharynx. They are flask-shaped and consist of elongated cells with their nucleii situated more towards their base than towards their centre. The free surface of the taste bud is generally sunk, giving the appearance of a gustatory pore. The undifferentiated cells occupy the remaining space of the epithelium. They vary in size and shape. Some of them are columnar and others cubical.

The sub-mucosa of the pharyngeal region of *P. canius* can be broadly divided into two parts. The narrow one, immediately next to mucosa, containing compactly arranged connective tissue, and the broader one, beneath it, having loose areolar connective tissue. The loose broader region contains in its meshes longitudinal muscle bands.

The muscularis-mucosa layer of pharynx consists of muscle bands, forming two coats. The longitudinal muscle bands which are found in the areolar connective tissue forms the inner coat of the musculature, whereas, the transversely disposed, circular muscle bundles occupy fairly a good portion of the thickness of the pharyngeal wall and form the outermost coat. Both these muscles are of striated type.

3.2.2 *Oesophagus* (figures 7a, b): The oesophageal wall is formed of four layers—mucosa, sub-mucosa, muscularis-mucosa and serosa. The mucosa formed of striated epithelium, is composed of mucous cells and undifferentiated cells. Taste buds are totally absent in this region. The epithelium of the anterior region is thicker than that of the posterior region of the oesophagus. The villi of the anterior region which are longitudinal and uninterrupted are blunt towards their tips and are of uniform size. The crypts are narrow. In the posterior region, the epithelium is thrown into folds and bear comparatively sharp edges which on meeting together almost fill up the lumen of the oesophagus. Mucous cells occur in large numbers in the posterior region of the oesophagus. Mucous cells of the oesophagus are like those of the pharynx in shape. The actively dividing undifferentiated cells are found towards the anterior part of the oesophagus.

The sub-mucosal region of the oesophagus consists of connective tissue fibres, loosely arranged, so as to form a meshwork. It is well vascularised and innervated. This tissue forms the central core for the folds of the oesophagus. It also forms a layer all around the epithelium.

The muscularis-mucosa layer of the anterior region of oesophagus is divisible into an inner longitudinal layer and an outer circular coat. The circular muscle fibres are transversely disposed and form a major part of the thickness of the wall of oesophagus. The muscle fibres of this region too are of striated variety.

The serosa is a conspicuous layer in this region, consisting of connective tissue fibres and cells, blood vessels and nerves, covered externally by flattened peritoneal cells. This coat of the oesophagus is external to circular muscle fibres and is continuous with the connective tissue found in this region.

Histologically observed structural details of oesophagus are a little different in the

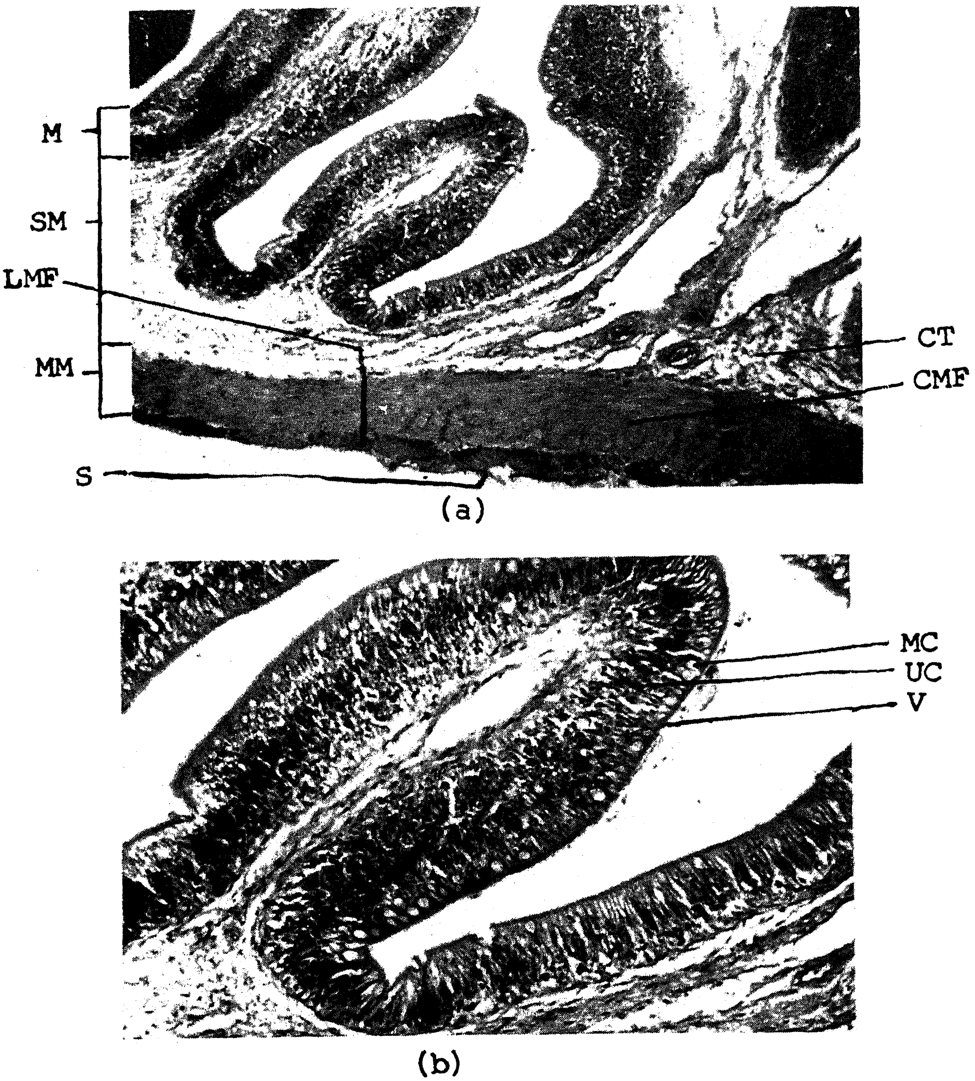


Figure 7. T.S. of oesophagus of *P. canius*. (a) × 40; (b) × 100.

posterior portion, where it passes into stomach. The main difference is the transformation of the stratified epithelial layer of mucosa into columnar epithelium. The submucosal layer diminishes in thickness and in the muscularis mucosa layer the position of the circular and longitudinal muscles are reversed, i.e. the inner layer is of circular muscle fibres and outer of longitudinal muscle fibres.

3.2.3 *Stomach* (figures 8 and 9): This part of the digestive tract can be structurally divided into an anterior cardiac region and a posterior pyloric region. Both the cardiac and pyloric stomachs are each formed of four usual tissue layers of mucosa, submucosa, muscularis-mucosa and serosa. The histological structure of the cardiac and

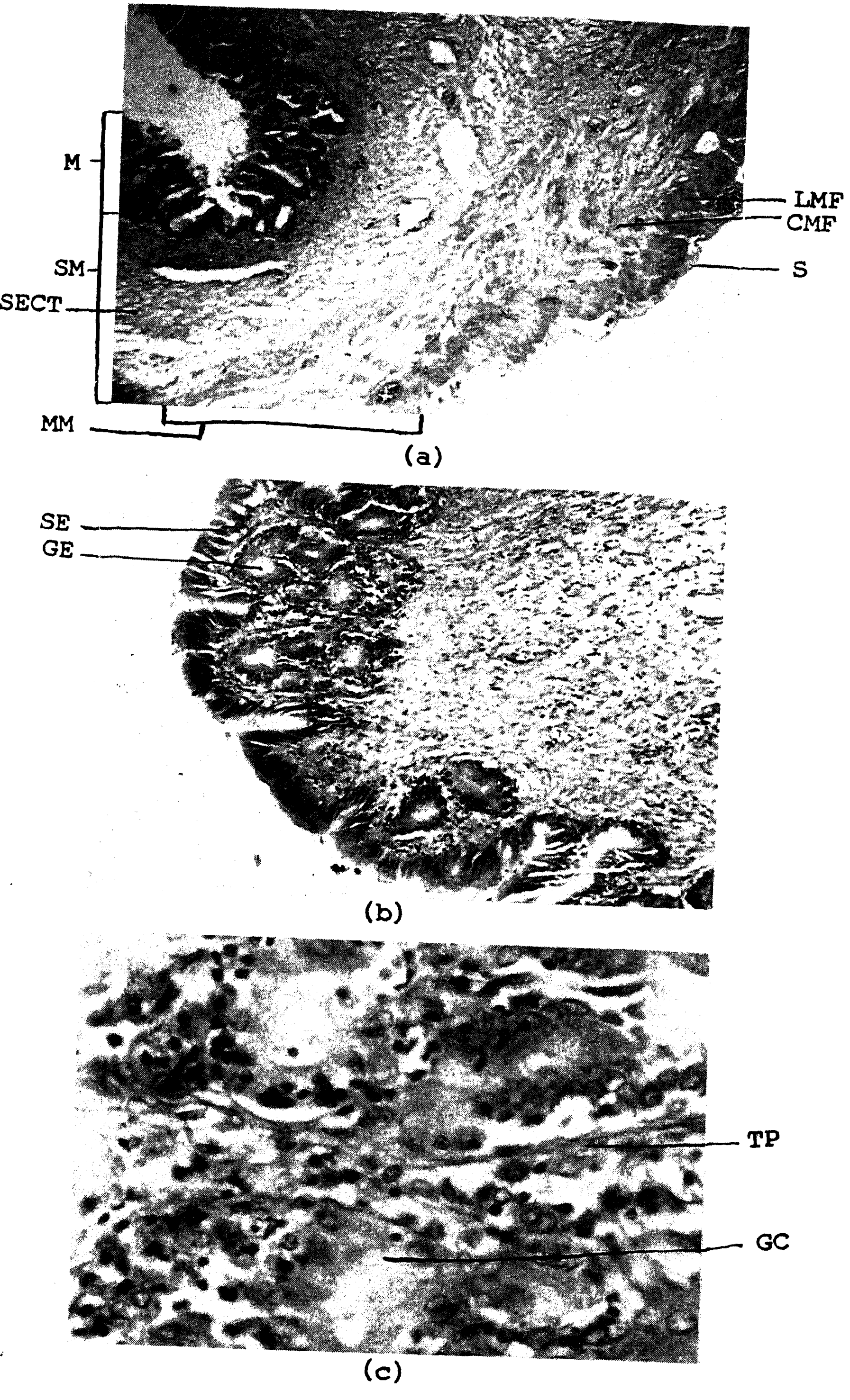


Figure 8. T.S. of cardiac stomach of *P. canius*. (a) × 40; (b) × 100; (c) × 400.

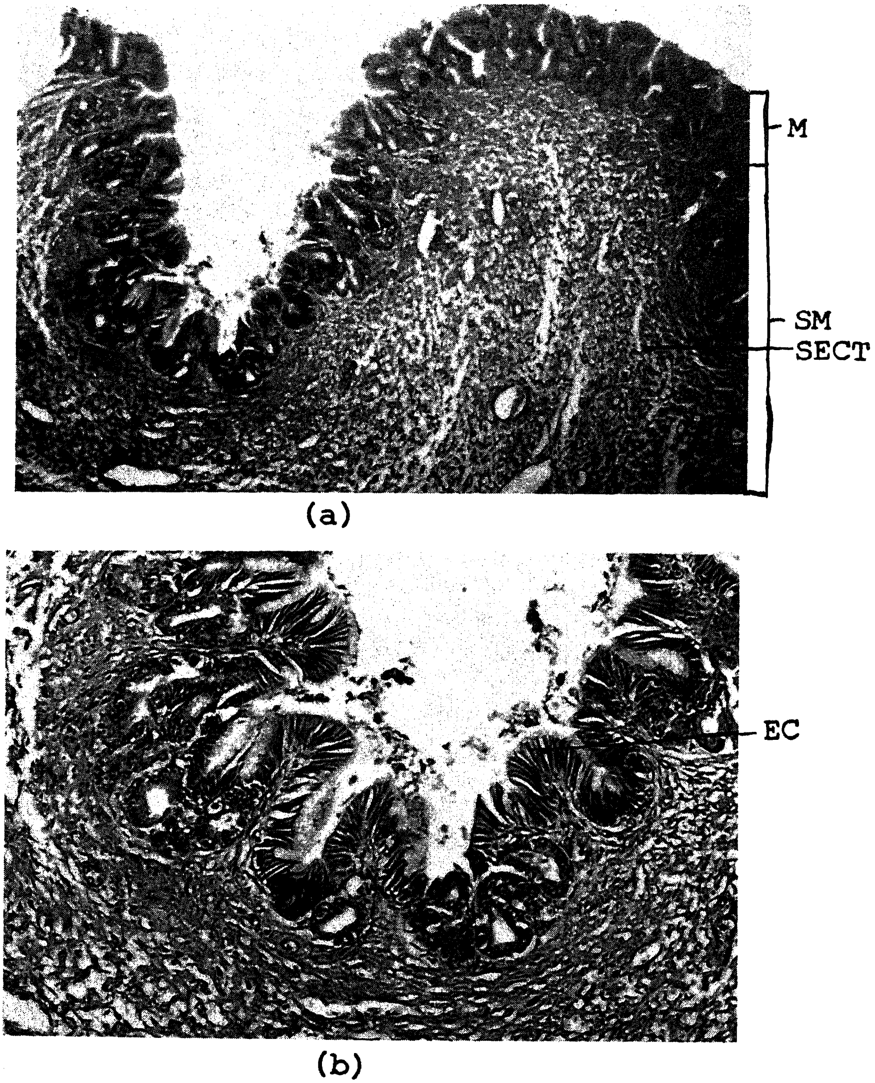


Figure 9. T.S. pyloric stomach of *P. canius*. (a) × 40; (b) × 100.

pyloric stomachs are similar in other layers but for the structure of the mucosal layer.

The mucosa of the cardiac stomach contains two types of epithelia, superficial and glandular. The superficial layer is made up of simple columnar epithelial cells which is thrown into a number of gastric folds. Each fold is supported by connective tissue fibres. The glandular layer of mucosa contains gland cells of the gastric glands which are of simple tubular type. Only one type of cell is seen amongst gland cells. This region is divided into compartments or blocks by the connective tissue septa found at regular intervals which connect the 'tunica propria' on one hand and the connective tissue, found beneath the gland cells, on the other.

The mucosa of the pyloric stomach form long villi, some of which may give rise to secondary foldings. It is made up of only one layer of epithelial cells of columnar type. Gland cells are absent in this region and so is 'tunica propria'.

The sub-mucosal layer of the stomach consists of sub-epithelial connective tissue. It is well developed in both the regions of the stomach. Not only does it form the central core of the gastric glands but also a circular layer internal to circular muscle fibre.

The muscularis-mucosa, in both the regions of the stomach, consist of thick internal circular muscle fibres and a thin external longitudinal muscle layer. The muscle fibres of the stomach are of non-striated type except in the region where oesophagus passes into stomach. The muscularis-mucosa layer of the stomach is thicker than the oesophageal and pharyngeal regions.

The serosa is of considerable thickness in this region and its structure is very much like that in the oesophageal region. At various places in the stomach wall the serosa is connected with the sub-epithelial connective tissue of sub-mucosal layer by connective tissue fibres.

3.2.4 *Intestine* (figures 10 and 11): The four usual tissue layers are present in this region as well. The mucosa is formed of simple, cylindrical columnar cells and is thrown into a number of folds which branch occasionally. In anterior portion of the intestine, the villi are longer and branched, whereas, in the posterior region they are unbranched and short. In addition to columnar cells, mucus-producing cells of the goblet type are also present in the mucosal layer of the intestine. Their number is much more in the posterior part of the intestine. The mucosal layer of the anterior intestine is quite broad and at places invaginated within the villi.

The sub-epithelial connective tissue, forming the sub-mucosal layer, forms the central core of the intestinal folds, as well as a thin layer internal to circular muscle fibres. This is richly vascularised and innervated.

The muscle fibres of the muscularis-mucosa layer are smooth and of non-striated type. These are arranged to form an internal circular muscle layer and an external longitudinal muscle layer. Longitudinal muscle fibre layer is comparatively thinner than the circular muscle fibre layer in this region. The circular muscle fibre layer is at places traversed by connective tissue layer of sub-mucosa. The muscularis-mucosa layer of the intestinal region is even thicker than that of pyloric stomach region.

The serosal layer is thickest in this region of the alimentary tract and its composition is much more like that seen covering the stomach region.

3.2.5 *Rectum* (figure 12): Tissue layers of the rectum are almost similar to that of intestine. Villi are fewer in number and are short and broad. Mucous cells are abundant. Muscularis-mucosa layer is comparatively thinner than that of intestine. Serosa forms a very thin layer in this region.

3.2.6 *Liver* (figure 13): The liver is enclosed in a thin covering of a fibrous connective tissue, the capsule, and is made up of characteristic polygonal hepatic cells with granular cytoplasm and spherical nucleii. Exocrine pancreatic acini lie embedded between the hepatic cells. Numerous patches of such pancreatic tissue are present. The exocrine cells are arranged in 1–3 rows, encircling the blood capillaries. The acini lying in the close proximity of the blood capillaries are columnar, whereas, the rest are pyramidal in shape. A typical exocrine cell contains a basal homogeneous region and an apical part, containing numerous zymogen granules.

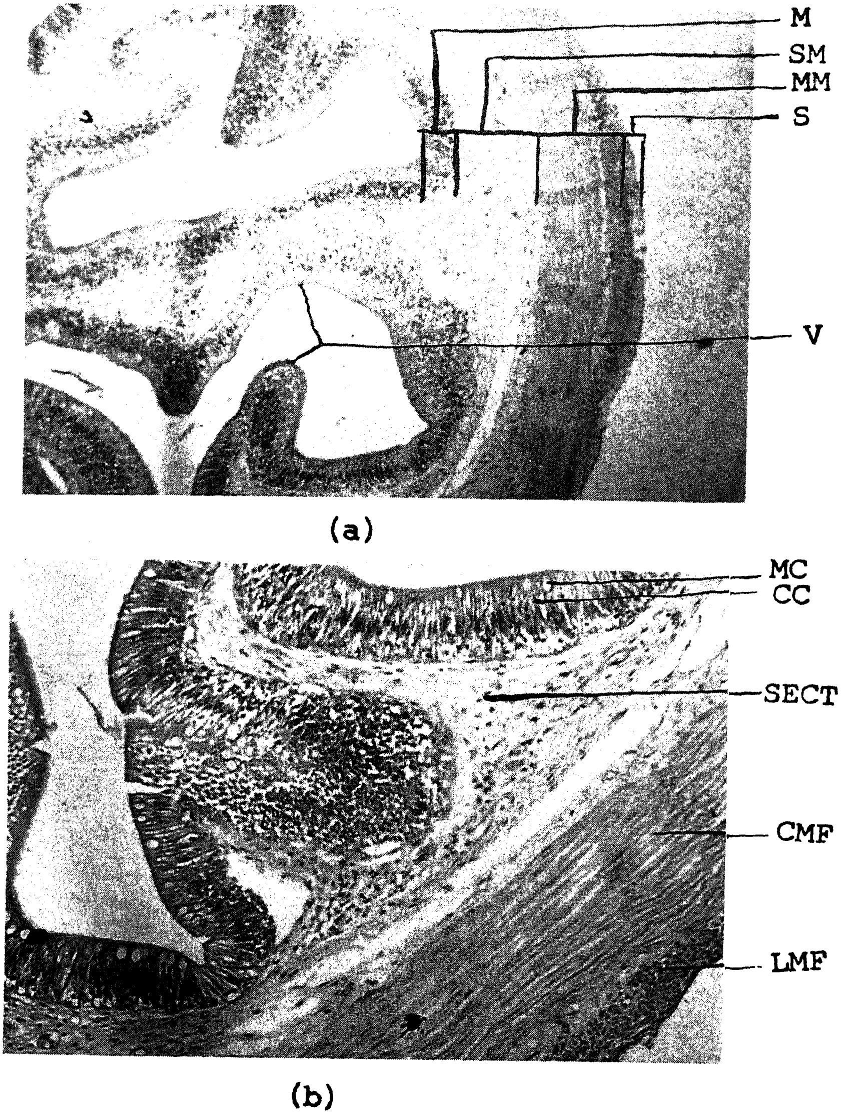


Figure 10. T.S. of anterior intestine of *P. canius*. (a) × 40; (b) × 100.

Islets of langerhans could not be located in the liver of *P. canius*. Any other pancreatic tissue, lying outside the liver, was also not found in this species.

4. Discussion

4.1 *Morphology and Anatomy*

The ventrally placed mouth of *P. canius* suggests a bottom feeding habit of the species. Khanna (1962) recorded a wide protractile mouth opening in the carnivorous fishes

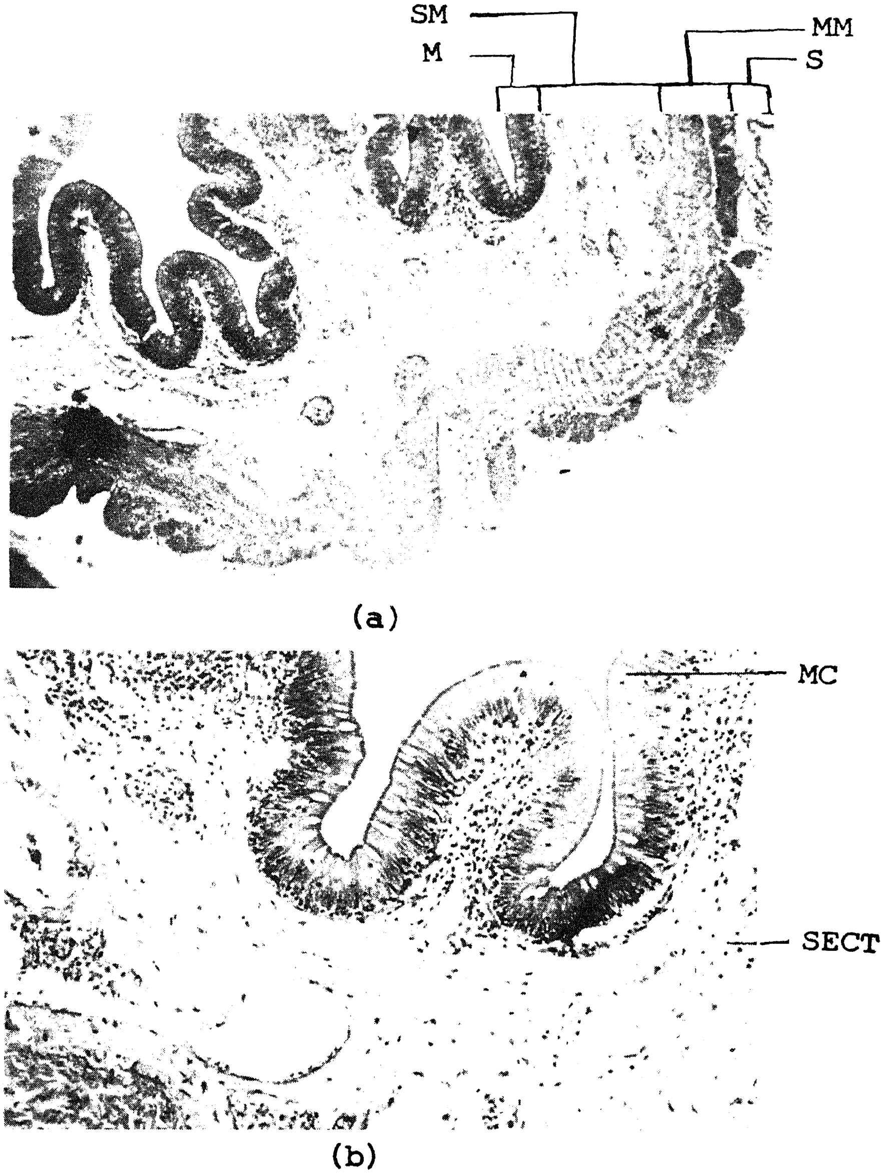


Figure 11. T.S. of posterior intestine of *P. canius*. (a) × 40; (b) × 100.

studied by him. The mouth in case of *P. canius* is transverse, non-protractile and comparatively small. The buccopharyngeal region bears small, sharp and pointed teeth on the maxilla and in the pharyngeal region, whereas, the vomerine and mandibular teeth are molar-like. According to Al-Hussaini (1947), pointed and conical teeth are commonly present in carnivorous predatory fishes. Several authors have reported the presence of well developed teeth in the buccopharyngeal region of carnivores (Das and Moitra 1956; Khanna 1962; Khanna and Mehrotra 1970). According to Das and Moitra (1956), the buccopharyngeal region of carnivorous fishes are provided with

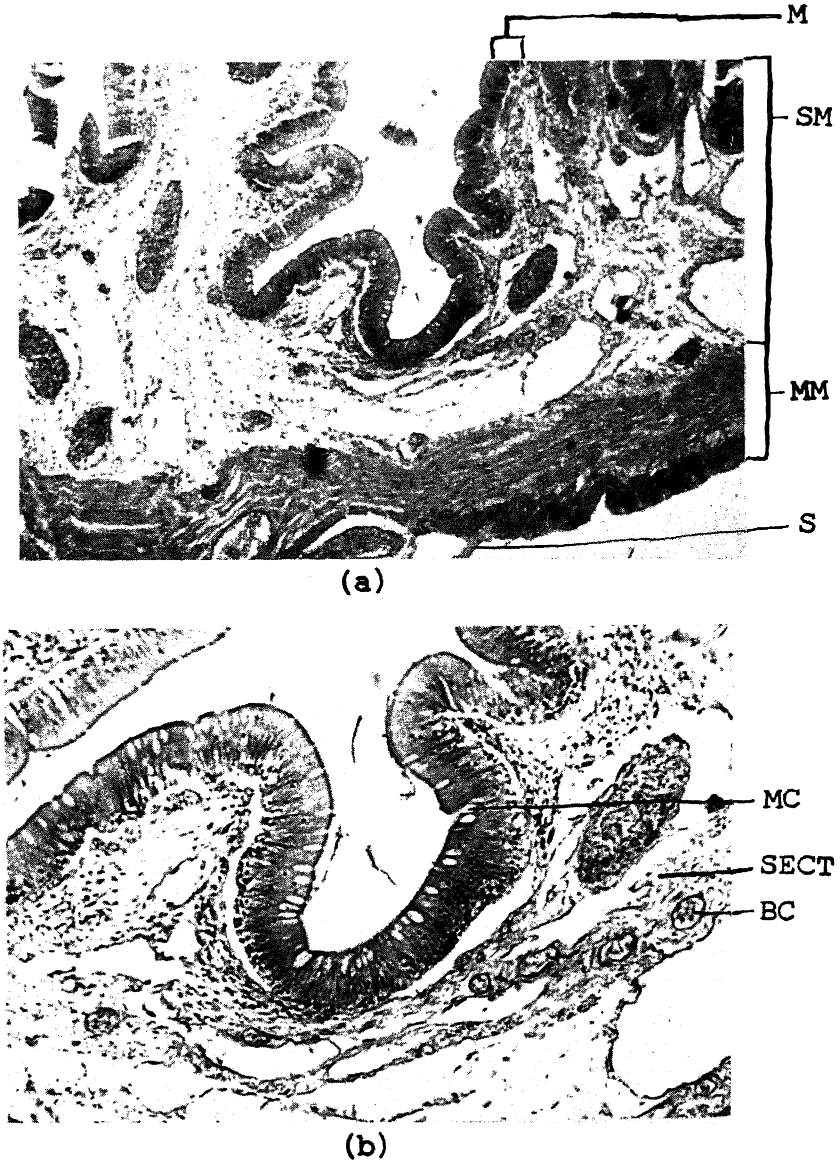
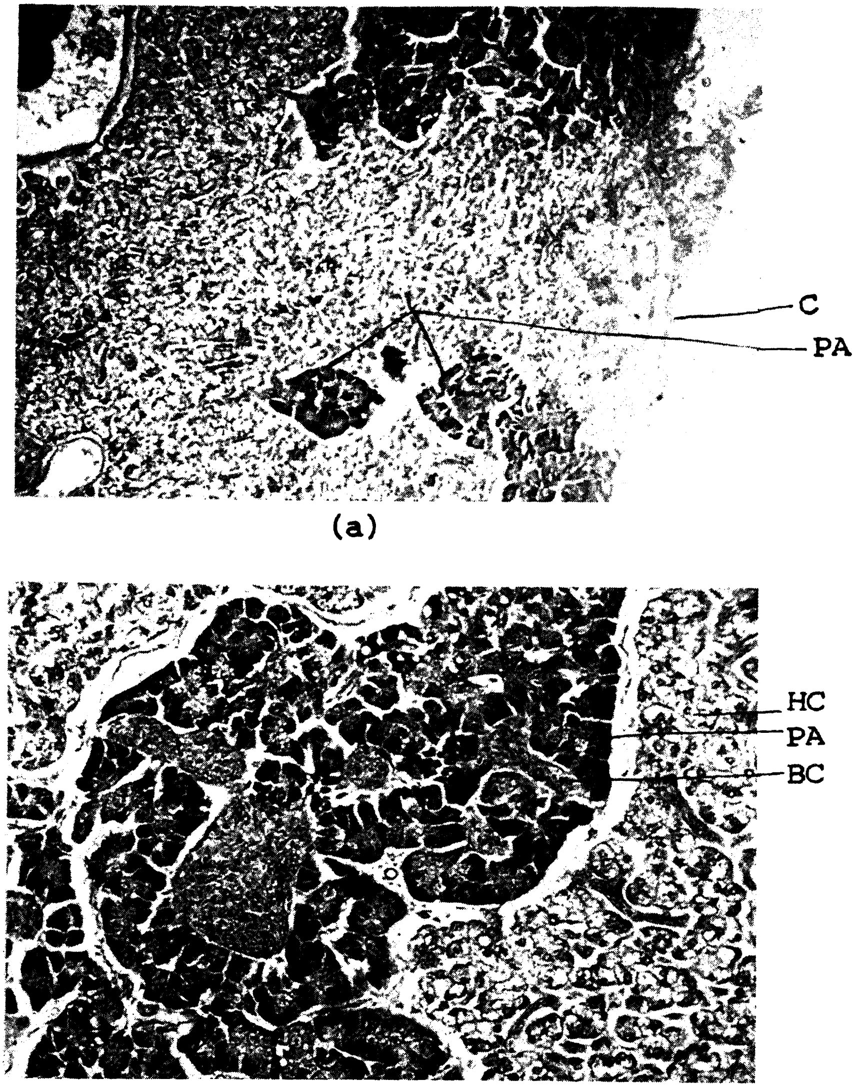


Figure 12. T.S. of rectum of *P. canius*. (a) × 40; (b) × 100.

highly specialised teeth for seizing, holding and tearing of ingested prey. The canine-like maxillary teeth of *P. canius* is suitable for tearing the prey, whereas, the molariform vomerine and mandibular teeth are probably meant for crushing. The conical teeth of pharynx and horny pad are so small that they can only perform the function of seizure of the prey with the help of the conical teeth-like gill-rakers of the third, fourth and fifth gill-arches. The structure of teeth in *P. canius*, thus, suggests that the food material enters the oesophagus after being crushed, unlike what is commonly found in other carnivore teleosts where the food is swallowed as a whole (Kapoor 1953; Barrington



(a)

(b)

Figure 13. T.S. of liver of *P. canius*. (a) × 40; (b) × 100.

1957; Mohsin 1961). This inference is also supported by the fact that the food material, broken up into small fragments, was invariably observed in the stomach of this species, during gut content analysis. An arrangement for crushing the food in case of *P. canius* is essential too, as both molluscs and crabs, the most preferred food item of the species (Sinha 1984), have hard exoskeleton which need to be broken to reach at the animal proper. Barrington (1957) was of the opinion that the provision of such a crushing arrangement may have a bearing in the absence of a true stomach in any carnivore.

Al-Hussaini (1947) described the gill-rakers as short or absent in the carnivorous fishes. Khanna (1962) and Kapoor (1965) believed that in carnivorous fishes the gill-rakers have been modified to form pointed teeth-like structures and supplement the teeth in macerating and preventing the escape of ingested prey. In *P. canius* it was observed that the first and second gill arches are provided with small gill-rakers. The size and number of gill-rakers is greatly reduced in the case of third, fourth and fifth gill-arches where they give more an appearance of conical-shaped teeth, set wide apart. These are, probably, employed for grasping and preventing the escape of the prey in association with other sets of teeth of the pharyngeal apparatus. It, thus, appears that the carnivorous nature of this species is responsible for such adaptive modifications in the structure of gill-rakers.

Venkateswarulu (1962) observed that structurally there is no difference in the nature of oesophagus of herbivorous and carnivorous fishes. But according to Mehrotra and Khanna (1969), carnivorous and piscivorous fishes preying on larger organisms, have generally larger distensible oesophagus. In the present study, it was observed that *P. canius* possesses a short oesophagus which has greater calibre in front. A large and distensible oesophagus is not required in this case too, as the food enters the oesophagus after being crushed. Sarkar (1959) also observed a short and narrow oesophagus in the carnivorous fish, *Mystus seenghala*.

P. canius lacks a true sac-like stomach, commonly found in carnivores. It is tubular in nature, being wider than oesophagus and intestine, its preceding and succeeding portions in the alimentary tract respectively. This type of stomach have been reported earlier also in many carnivores (Das and Moitra 1956; Venkateswarulu 1962; Moitra and Ray 1979).

It is generally known that the intestine is the longest in herbivores and shortest in carnivores with intermediate values in omnivores (Das and Moitra 1956; Sinha 1972; Kapoor *et al* 1975). The intestine of *P. canius* though convoluted, is comparatively short with the gut length—total length of fish ratio ranging between 0·78–1·19 (av. 1·08). According to Khanna (1962), the type of food ingested by a fish primarily influences the length of the gut. Barrington (1957) was, however, of the opinion that more than one factor is responsible for determining the ratio between the length of the gut and total length of the fish. Al-Hussaini (1949) showed that the shortness of the gut in a fish may be compensated by the increase in the complexity of its mucosal folds and, thus, the length of the gut is affected by its average mucosal area. In *P. canius* the mucosal folds of the intestine are of complex nature, having longitudinal as well as reticular bands in the anterior portion, which appear to have probably evolved to compensate for reduction of intestinal length, so as not to affect the total absorptive area.

The liver is the principal digestive gland in *P. canius*. Slipper (1946) classified the distribution of pancreas in teleosts in five types: pancreas compactum, pancreas diffusum, pancreas disseminatum, pancreas juxtoahepaticum and pancreas hepaticum. Pancreatic tissue in *P. canius* were observed to be present in a primitive diffused state, lying only within the hepatic tissue in a dispersed condition. No other type of pancreatic tissue could be recognised in this species. It can, thus, be said that only 'pancreas hepaticum' of Slipper (1946) was observed in *P. canius.*

4.2 *Histology*

Bhimachar (1935) divided fishes into three groups according to their mode of feeding, viz those feeding by sight only, those feeding by taste and those feeding by both sight and taste. Khanna (1968) opined that the taste buds are rare or absent in fishes which fed by sight only and few taste buds are present in those which fed by sight and taste, while in fishes feeding by taste only, abundant taste buds are present. Pasha (1964) concluded that carnivorous fishes do not require a well organised sense of gustation. The buccopharynx which is lined by stratified epithelium and is richly supplied with mucous cells and often with taste buds, has been assigned the function of seizure, control and, probably, selection of food by Barrington (1957). Kapoor *et al* (1975) regarded the main function of this region to be mucus production and gustation. In *P. canius* it has been observed that taste buds are very few, present only upto the posterior part of the pharynx and are poorly developed. They are entirely absent from oesophagus downwards. Similar observations have also been made by Mehrotra and Khanna (1969) and Moitra and Ray (1979) in the carnivores studied by them. This pattern of distribution of taste buds in *P. canius* indicates that this has only a secondary importance in food selection in this species. This job, probably, being primarily done through sight and four pairs of barbels encircling the mouth (figure 2).

In *P. canius* mucous cells are few in buccopharyngeal region, with their concentration gradually increasing posteriorly. They are much more abundant in the oesophageal region. They are comparatively bigger in size too in this region. The stomach region is again devoid of mucous cells with their number again increasing in the intestinal region, posteriorly. The maximum concentration of mucous cells is in the posterior region of the intestine and the rectum. The mucous cells secrete mucus for binding together the coarser food particles to form the bolus for onward transmission along the gastro-intestinal tract (Al-Hussaini 1949) and also for protection of the soft epithelial lining from mechanical injury (Moitra and Sinha 1972). The mucous cells of the buccopharyngeal region, as well the oesophagus, of *P. canius* are, probably, responsible for mucus production to lubricate the food for easy swallowing into the stomach, a purpose assigned to mucous cells by Al-Hussaini (1949). The fewer mucous cells in the anterior intestine of *P. canius*, is probably because the need for lubrication of the food is minimised in the anterior intestinal region, as it enters the said region in a comminuted and semidigested condition. Fewer number of mucous cells in anterior intestinal region may also be for the fact that excess of mucus in this region would weaken the digestive juices as well as impair absorption. The posterior intestine has more number of mucous cells, probably for lubricating the undigested food material for onward progression into the rectum. The mucous cells attaining maximum concentration in the rectal mucosa, is obviously to facilitate defaecation (Al-Hussaini 1949).

The mucosal layer of the buccopharynx and oesophagus is made of stratified epithelium, whereas, stomach downwards is of columnar epithelium. The superficial layer of columnar epithelium of cardiac stomach has been described to be absorptive in function (Blake 1936). Al-Hussaini (1946), however, considered these cells to be responsible for secreting mucus too, where no mucous cells are found in this region. In the present case, it may, thus, be said that columnar epithelial cell of the stomach region perform dual function of secretion of mucus and absorption of digested food materials, since no mucous cells are found in this region in this species. An identical function of the superficial epithelium of the gastric region has also been described by Mehrotra and Khanna (1969) and Moitra and Ray (1979) in carnivores studied by them. The columnar epithelium cells of the intestine are only responsible for absorptive function since the mucus production function here is taken up by specific mucous cells.

A large number of gastric gland cells which are simple and of tubular type are present in the glandular epithelium of the cardiac stomach of *P. canius*. Unlike in higher vertebrates, only one type of cell was observed in the gastric gland of this species. Only one type of gland cell in the gastric region has been reported by Blake (1936), Sarkar (1959), Pasha (1964), Mehrotra and Khanna (1969) and Moitra and Ray (1979) in several other species. Hence, it can be inferred that the gastric glands of *P. canius* show a more primitive organisation than in higher vertebrates.

In *P. canius*, the villi of the anterior intestinal region show secondary foldings and branchings. Khanna and Mehrotra (1971) stated that such foldings may help to increase the total absorptive surface area of the intestine. Al-Hussaini (1949) was of the opinion that these secondary foldings and branchings are more in fishes with shorter intestine to ensure optimum absorptive surface.

The wall of the pharynx and oesophagus are comprised of inner longitudinal muscle fibres and outer circular muscle fibres, whereas, stomach downwards the position is reversed, in this species. Such an arrangement of the muscle layers appears to be associated with the carnivorous habit of the species. Whereas, the outer circular and inner longitudinal muscle layers in the pharynx and oesophagus provide additional strength to the mucosal folds and increase their elasticity to facilitate the swallowing of the larger organisms, the reverse arrangement of the muscle layers in stomach and intestinal regions aids to the elasticity of their walls within specific limits.

Striated muscles are known to be voluntary in nature, whereas non-striated are involuntary. The presence of striated muscle fibres in the region of pharynx and oesophagus in *P. canius* must be to help the fish in regurgitation or voluntary swallowing of the food. No such function is to be served in the region of stomach downwards, where the food is to simply move onwards with the peristaltic movement and thus, the muscles here are of non-striated type.

The pancreas in *P. canius* occurs in primitive diffused condition and remains embedded within the intra-hepatic tissue. Similar pancreatic cells have been reported in other fishes by Moitra and Ray (1979). Islets of Langerhans could not be located in the pancreas of *P. canius*, a feature also reported by Moitra and Ray (1979) in fishes studied by them.

It can, thus, be inferred from the above studies that the digestive system of *P. canius* is of a carnivore, with characters of both primitive as well as developed carnivorous nature. The narrow, non-protractile mouth, lack of true sac-like stomach—a feature uncommon for a true carnivore, and the total length of the fish—total gut length ratio of near unity are all suggestive of its primitive carnivorous nature, whereas, the canine-like maxillaries and molariform vomerine and mandibular teeth, very well suited for tearing and crushing of the prey respectively, are suggestive of a well developed carnivorous nature. The food preference study of the species (Sinha 1984) also suggested predatory carnivorous habit of the fish which was evident right from its fingerling stage. The two studies of food preference and morphology, anatomy and histology of the alimentary canal and its associated structures, together complement each other and establish *P. canius* as a predatory carnivore which preys on aquatic animals of small size.

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Abbreviations used in figures: ACT, Areolar connective tissue; BC, blood capillary; C, capsule; CC, columnar cell; CMF, circular muscle fibre; CT, connective tissue; EC, epithelial cells; GC, gland cell; GE, glandular epithelium; HC, hepatic cell; LMF, longitudinal muscle fibre; M, mucosa; MC, mucous cell; MM, muscularis mucosa; PA, pancreatic acini; S, serosa; SE, superficial epithelium; SECT, sub-epithelial connective tissue; SM, sub-mucosa; TB, taste bud; TP, tunica propria; UC, undifferentiated cell; V, villi.

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Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats

P A DECHAMMA, S KAMATH and P S SHETTY

ICMR Nutrition Research Centre, Department of Physiology, St. John's Medical College,
Bangalore 560 034, India

MS received 6 May 1985

Abstract. Interscapular brown adipose tissue was studied in pregnant and lactating females and compared with virgin females in inbred Wistar rats, using the histomorphology and quantitation of cell numbers. In pregnant females an increase in the interscapular brown adipose tissue weight, total fat content and total fat cell numbers associated with a decrease in adipocyte diameter was observed when compared with virgin females. The total lipid content and total fat cell number in the interscapular brown adipose tissue of lactating females showed a decrease when compared to the pregnant females. This suggests that pregnancy and lactation are accompanied by changes in the status of interscapular brown adipose tissue which may be related to the changes in energy metabolism in these physiological states.

Keywords. Interscapular brown adipose tissue; adipocyte histomorphology; adipocyte size; adipocyte number.

1. Introduction

Brown adipose tissue (BAT) is an important organ responsible for non-shivering thermogenesis in cold-adapted mammals (Afzelius 1970) and for the maintenance of the core-body temperature (Foster and Frydman 1978). It may also regulate energy balance (Rothwell and Stock 1981) and participate in the regulation of body weight (Cannolly *et al* 1982), since the role of BAT mediated thermogenesis in the regulation of body weight and energy stores is now considered a credible hypothesis (Trayhurn and James 1981). Further support is forthcoming from studies on pregnant and lactating animals which imply that conservation in energy expenditure are achieved in these physiological states by altering the activity of BAT metabolism (Trayhurn *et al* 1982).

In the present study, inbred Wistar rats were used to assess the functional status of interscapular brown adipose tissue (IBAT) in pregnant, lactating and virgin females, using histomorphology, adipocyte size and number. A preliminary study was also carried out to find out whether there were any differences in the IBAT of males and females.

2. Materials and methods

Inbred Wistar rats were used for studying IBAT in the different animal models, i.e., males, virgin females, pregnant and lactating females. Three month old rats, with body weight range between 150–250 g were chosen for the study. The animals were maintained in the animal house at 25 + 2° C and were given food and water *ad libitum*.

The animals were sacrificed using ether and the IBAT was excised. It was trimmed off the surrounding skeletal muscle and white adipose tissue, blotted dry and the weight recorded. One half of the IBAT was used for total lipid estimation using the method of Folch *et al* (1957). From the other half, a portion was fixed in Bouin's fluid, processed and embedded in paraffin wax for histological observations after staining with haematoxylin-eosin (H-E) or Azocarmine-G.

The remaining bit of IBAT was immediately frozen to −20°C in the cryostat. 80–100 μ thick frozen sections were cut for the adipocyte diameter measurement. The sections were taken out from the cryostat knife using a coverslip and mounted on a slide with a drop of glycerine. The diameter of the adipocytes were measured according to the method of Ashwell *et al* (1976) at a magnification of × 400 using an ocular micrometer. From each section 10 adipocytes were measured horizontally and 10 vertically. Diameters were recorded in 20 sections per IBAT specimen; a total of 400 cells were measured. The divisions on the ocular micrometer were calibrated using a Leitz stage micrometer. The adipocyte diameter was measured in the units of the ocular micrometer and then converted into microns.

2.1 *Calculations*

The total fat cell number was calculated using the formula (Trayhurn *et al* 1979):- $\mathrm{MCV} = (3\mathrm{SD}^2\ \mathrm{MCD} + \mathrm{MCD}^3)/6$ (MCV = mean cell volume; MCD = mean cell diameter; SD = standard deviation of the adipocyte diameter). The mean fat cell mass (MFCM) was calculated by multiplying MCV by the density of the triglyceride (0·915) that the cell contained. The total fat cell number was derived by dividing the total fat in the depot estimated chemically by the mean fat cell mass.

3. Observations

3.1 *Weight of IBAT*

The weight of the IBAT in males was significantly higher than that of virgin females, however, the IBAT weight per unit body weight was significantly greater ($P < 0{\cdot}01$) in virgin females. A significant increase ($P < 0{\cdot}01$) in the total IBAT weight was noticed in the pregnant females as compared to the virgin females, although the IBAT weight per unit body weight did not significantly differ. The IBAT weight of lactating females was less than that of virgin and pregnant females, however, the decrease in weight was more significant when compared with pregnant females (table 1).

3.2 *Histology and histomorphology*

In males and virgin females the IBAT showed some adipocytes with multilocular lipid droplets and others without lipid droplets. The latter cells were mainly observed surrounding the blood vessels (figure 1), and had a peripherally situated spherical nucleus (figure 2). In pregnant females the cells were predominantly brown adipocytes with multilocular lipid droplets. The adipocytes had spherical nuclei which were displaced

Table 1. Showing body weight, IBAT weight and IBAT wt/unit body weight in inbred Wistar rats.

Group	Body weight (g)	IBAT weight (mg)	IBAT weight/ unit body weight
Virgin females (15)	158·0 ±5·6	158·3 ±8·9	101·2 ±5·6
Pregnant females (13)	230·7 ±6.6	212·9 ±9.4^{c}	92·9 ±4·2NS
(7)	195·4 ±7·1^{e}	203·6 ±7.1^{c}	105·3 ±5·8NS
Lactating females (11)	197·7 ±6·1	121·8 ±9·3^{a,d}	60·1 ±4·1^{c,d}
Males (12)	271·0 ±8·5	199·0 ±10·8^{b}	75·0 ±5·3^{b}

Number in the parenthesis indicates the number of animals used.
±Standard error of the mean.
$^{a}P < 0·01$;
$^{c}P < 0·001$—when compared to virgin females.
$^{d}P < 0·001$—when compared to pregnant females.
^{e}Weight on day 19th excluding weight of pups, amniotic fluid etc.
NS = Not significant.

to the periphery. The adipocytes without lipid droplets were few (figure 3). In lactating females however, the majority of adipocytes were devoid of lipid droplets. The tissue had a classical ‘worm-eaten’ appearance (Afzelius 1970). The number of adipocytes with lipid droplets were reduced (figure 4).

3.3 *Diameter of the adipocytes*

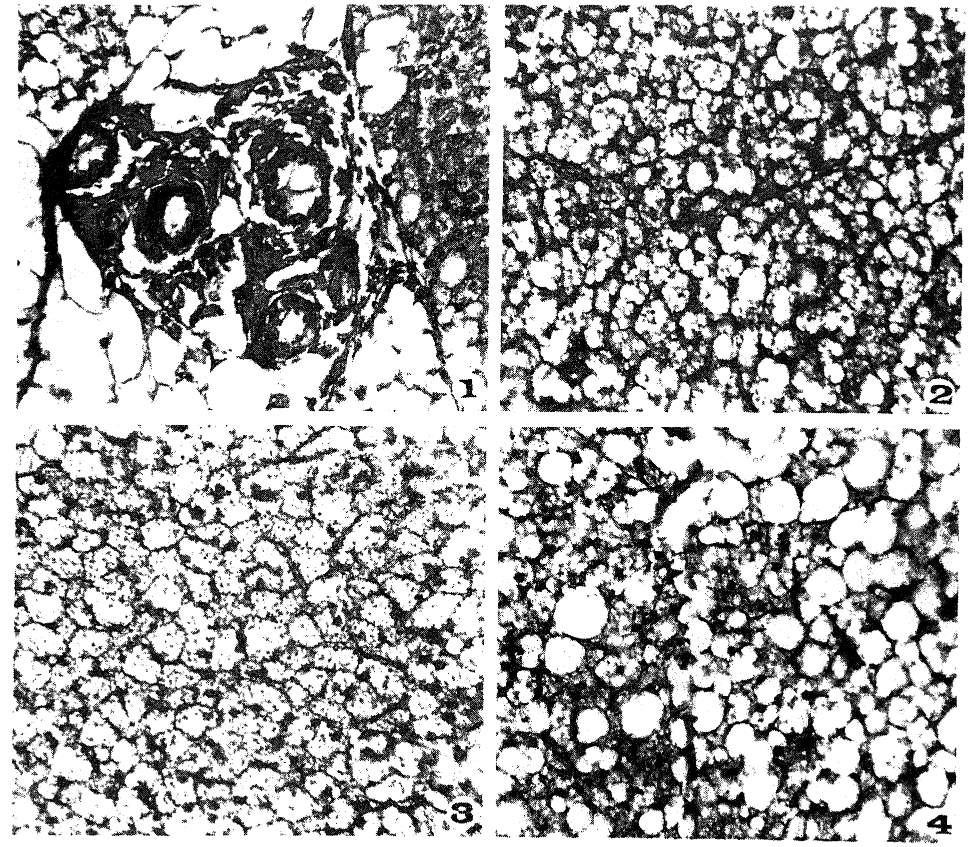
The adipocyte diameter of males and virgin females did not show any significant differences. Pregnant females showed a significant decrease ($P < 0·001$) in adipocyte diameter when compared to the virgin females. The adipocyte diameter of the lactating females was also significantly lower ($P < 0·01$) than in the virgin females. On the other hand, the adipocyte diameter of the lactating females was significantly higher ($P < 0·05$) when compared to pregnant females (table 2).

3.4 *Total lipid content*

No significant difference was seen in the total lipid content of IBAT of males and virgin females. In pregnant females the total lipid content was significantly higher ($P < 0·02$) than that of virgin females. The total lipid content in lactating females did not show any significant difference when compared with virgin females, but showed a significant decrease when compared to pregnant females (table 2).

3.5 *Total fat cell number*

There was no significant difference in the estimate of total fat cell numbers of males and virgin females. A statistically significant 3-fold increase in the total fat cell number was



Figures 1–4. Photomicrographs of IBAT in the different animal models. Bouin, 5μ Azocarmine-G. **1.** Section of IBAT in male-adipocytes around the blood vessels depleted of lipid droplets. × 250. **2.** Section of IBAT in virgin females-showing adipocytes with lipid droplets and without lipid droplets. × 250. **3.** Adipocytes in pregnant females with multilocular lipid droplets and spherical nucleus. × 250. **4.** Adipocytes in lactating females devoid of lipid droplets. × 250

Table 2. Showing the cell diameter, total lipid content and total fat cell number in IBAT in inbred Wistar rats.

Group	Cell diameter (μ)	Total lipid quantity (mg)	Total fat cell number × 10^6
Virgin females (15)	36·52 ± 1·35	60·73 ± 5·07	2·2 ± 0·38
Pregnant females (7)	28·34 ± 0·56^{d}	90·05 ± 0·95^{b}	6·7 ± 0·7^{d}
Lactating females (9)	31·01 ± 0·86$^{c, e}$	50·61 ± 6·86^{f}	3·1 ± 0·33$^{a, g}$
Males (12)	34·74 ± 1·56	60·04 ± 8·96	2·8 ± 0·37

Numbers in the parenthesis indicate the number of animals used.

± = Standard error of the mean.

$^{a}P < 0{\cdot}05$; $^{b}P < 0{\cdot}02$; $^{c}P < 0{\cdot}01$; $^{d}P < 0{\cdot}001$—when compared with virgin females.

$^{e}P < 0{\cdot}05$; $^{f}P < 0{\cdot}01$; $^{g}P < 0{\cdot}001$—when compared with pregnant females.

observed in pregnant females when compared with virgin females. However, the total fat cell number was lower in lactating females than in pregnant females (table 2).

4. Discussion

Brown adipose tissue is known to undergo changes in the two physiologically hyperphagic situations of pregnancy and lactation. Rothbard (1958) reported the hypertrophy of brown fat in pregnant mice shortly before term and a rapid depletion of lipid in the fat cells just prior to and at the time of delivery. In bats, *Tadarida b. Mexicana* it was reported that the lipid in the brown fat cells start accumulation in early pregnancy and reaches a maximum level late in the gestation period. After parturition, the brown adipose tissue is essentially depleted of lipid (Sims *et al* 1962). The findings of this study are consistent with these reports, since maximum lipid accumulation was observed during late pregnancy which resulted in hyperplasia of the IBAT. Histomorphology too, revealed that the majority of the adipocytes were filled with lipid droplets. On the other hand, depletion of total lipid was observed in the lactating females accompanied by the reduction in the total fat cell number. There was a tendency for the adipocyte number to revert back to the level of that of virgin females.

The increase in IBAT parameters in pregnancy is said to be due to the effects of adrenal hypertrophy (Sims *et al* 1962). However, other endocrine glands may be involved since a gradual depletion of lipid accompanied by weight reduction of brown fat of hamsters was observed as the gestation period advanced (Teodoru and Grishman 1959). Agius and Williamson (1980) reported that lipogenesis increased 5-fold during pregnancy and decreased at parturition and mid-lactation. Trayhurn *et al* (1982) have pointed out that in pregnant and lactating mice an increase in the IBAT weight resulted as there was 'suppression of thermogenesis', and further stated that this might be an adaptation for energy conservation.

On the basis of these two reports (Agius and Williamson 1980; Trayhurn *et al* 1982) the present study suggests that there is either a decreased rate of lipolysis or an increased rate of lipogenesis in pregnancy. In lactating females the depletion of lipid stored during pregnancy may be due to its utilization for the production of milk and to keep the pups warm by way of increased heat production. The present study also gives the histomorphological and quantitative evidence for the physiological status of the IBAT during pregnancy and lactation.

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Effect of ethylestrenol on growth and food utilization in *Channa striatus*

V ARUL

School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. Juveniles of *Channa striatus* were fed twice a week with hormone supplemented diet at concentrations of 0, 1, 2·5, 5 or 10 mg/kg fish and the hormone effects on growth and food utilization were observed for a period of 75 days. Neither ethylestrenol nor the tested doses of the steroid appear to significantly change feeding rate. Administration of 5 mg of ethylestrenol/kg fish not only enhanced growth but also conversion efficiency.

Keywords. Steroid supplementation; ethylestrenol; appetite stimulant; anabolic steroid; conversion efficiency; *Channa striatus*.

1. Introduction

From a large number of recent publications on application of growth hormone in fish culture, it has been possible to identify 14 androgenic steroids, which were found to stimulate growth in fish (Donaldson *et al* 1979; Higgs *et al* 1982). However the response to each of these steroids is species specific; for instance, 17α-methyltestosterone promotes growth in *Oncorhynchus kisutch* (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1975, 1977, 1979). *Cyprinus carpio* (Lone and Matty 1980), *Tilapia aurea* and *Tilapia mossambica* (Guerrero 1975, 1976) but inhibited growth in *Salmo salar* (Simpson 1976) and *Salmo gairdneri* (Sower 1978). Conversely these two species positively respond to the treatment of ethylestrenol an anabolic androgenic steroid (Simpson 1976; Ince *et al* 1982; Lone *et al* 1982).

In India effects of growth hormones was tested mostly in air-breathing fishes (Nirmala and Pandian 1983; Sindhu and Pandian 1984). However, these authors have restricted the number and the duration of the experiment; further the hormone was injected on the first day of experiment. It is known that application of growth hormone through injection involves tedious labour in aquaculture practice. Secondly as the injected hormone gets rapidly metabolised and eliminated (Donaldson *et al* 1979), the desired positive response may not persist long. Hence long term studies on the effect of hormone, supplemented at lower doses through food are required. The present long term study was undertaken to ascertain the response of *Channa striatus* fed on supplemented diet with ethylestrenol.

2. Materials and methods

Juveniles of *C. striatus* (9·4 ± 0·41 g) were acclimated to laboratory conditions and divided into 5 groups. They were reared individually in cylindrical aquaria (14 L capacity) for 75 days under natural L/D cycle (14/10 hr). Room temperature averaged 28 ± 2°C. The aquarium water was changed every 3 days. At the start of the experiment and thereafter every 15 days, the test individuals were weighed to the nearest 1 mg in a 'Stanton' top pan balance.

The test animals were fed at 09·00 hr on weighed amounts of live *Lepidocephalichthys thermalis*, which were left in the aquaria for a maximum period of 2 hr, i.e. as it would happen in the natural habitat, the prey had to be actively predated by the fish. The uneaten prey remains were collected subsequently with a hand sieve and dried to weight constancy at 90°C for 6 hr. Faeces was collected by filtering the aquarium once in 3 days. The sacrifice method (Maynard and Loosli 1962) was followed to determine water and energy contents of the test individuals at the beginning of the experiment. Energy contents of food, faeces and test animals were estimated in a Parr 1411 Semi-micro bomb calorimeter.

The energetic equation in the present study is that of IBP formula (Petrusewicz and MacFadyen 1970) represented as: $C = F + U + P + R$; where C is the food consumed, F the faeces, U the nitrogenous excretory waste, P the growth and R the energy released as heat due to metabolism. Rates of feeding, absorption and conversion were calculated using the following formulae:

$$\text{Feeding rate (Cr) (J/g/day)} = \frac{\text{Average food consumed (J/day)}}{\text{Mid-body weight (g)}}$$

$$\text{Absorption rate (Ar) (J/g/day)} = \frac{\text{Average food absorbed (J/day)}}{\text{Mid-body weight (g)}}$$

$$\text{Conversion rate (Pr) (J/g/day)} = \frac{\text{Average food converted (J/day)}}{\text{Mid-body weight (g)}}$$

$$\text{Absorption efficiency (\%)} = \frac{\text{Food absorbed (J)}}{\text{Food consumed (J)}} \times 100$$

$$\text{Conversion efficiency (\%)} = \frac{\text{Food converted (J)}}{\text{Food absorbed (J)}} \times 100 .$$

Mid-body weight of the test animal is the mid-point in the growth curve of the animal for the test period. It was calculated by the following formula:

$$\text{Mid-body weight} = \frac{\begin{array}{c}\text{Initial live weight}\\ \text{of the test fish (g)}\end{array} + \begin{array}{c}\text{Final live weight of}\\ \text{the test fish (g)}\end{array}}{2}$$

2.1 *Hormone administration*

The selected dosages of the hormone ethylestrenol (1, 2·5, 5 and 10 mg/kg fish) were dissolved in sesame oil (carrier solution). Hormone supplementation of diet was done by intramuscularly injecting the chosen dosage of the hormone into the prey organism *L. thermalis*, starved for 24 hr (to ensure the evacuation of gut), before being offered as food for the test individuals. As soon as the hormone treated *L. thermalis* was released into an aquarium, the fish consumed the entire prey, hence the hormone injected into the prey was received by the test individuals without any loss. After ensuring that *C. striatus* has consumed the entire prey supplemented with hormone, the remaining portion of the weighed *L. thermalis* was offered. The control fish was fed with *L.*

thermalis injected with sesame oil alone. Volume of the hormone solution injected was maintained constant at 25 μl for all the tested dosages. For injection, a 25 μl capacity sterilised tuberculin syringe (Top syringe, Bombay) was used.

The hormone supplemented diet was fed to the fish twice a week for a period of 75 days. Once in 15 days the quantity of the hormone injected into the prey organism was adjusted with new mean weight of the fish. All data were subjected to statistical analyses such as students test and single way analysis of variance.

3. Results

3.1 *Consumption*

Feeding rate of individuals of *C. striatus* in the sham-treated group averaged to 228 Joules/g live fish/day. Neither ethylestrenol nor the tested doses of the steroid appear to significantly ($P > 0{\cdot}05$, table 1) change the feeding rate, which averaged to 228 J/g/day in the 4 treated groups. Hence it may be said that ethylestrenol is not an appetite stimulant for *C. striatus*.

3.2 *Absorption*

Trends obtained for the absorption rate of fish receiving different dosages of ethylestrenol followed that of feeding rate. Control group absorbed the food at a rate of 211 J/g/day. No difference was observed among the treated individuals, and also between control and treated ones. However the tested dosages of ethylestrenol (1, 2·5, 5 and 10 mg/kg fish) increases the absorption efficiency to 96 % from 92 % in the control. These were also in consistent with values obtained by the method of Pandian and Marian (1985). The minor differences among the hormone treated individuals were not statistically significant ($P > 0{\cdot}05$), but the difference between treated and sham groups was highly significant ($P < 0{\cdot}01$).

Table 1. Bioenergetics of *C. striatus* fed on diets supplemented with different dosages of the steroid hormone ethylestrenol. Each value ($\bar{X} \pm \mathrm{SD}$) represents the average performance of 3 individuals fed for a period of 75 days. Rates are expressed in J/g live fish/day and the efficiencies in percentage.

	Concentration of hormone (mg/kg fish)				
Parameters	0	1	2·5	5	10
Consumption rate	228 ± 17·0	236 ± 6·0	232 ± 14·0	224 ± 18·0	221 ± 14·0
Absorption rate	221 ± 16·0	225 ± 5·0	222 ± 10·0	214 ± 20·0	214 ± 14·0
Production rate	48 ± 5·0	79 ± 5·0*	78 ± 2·0*	83 ± 4·0*	69 ± 6·0*
Absorption efficiency	93 ± 1·0	96 ± 0·3*	95 ± 0·4*	96 ± 0·1*	96 ± 0·5*
Net conversion efficiency	23 ± 1·0	35 ± 2·0*	35 ± 1·8*	40 ± 0·7*	27 ± 2·0*

Values with asterisk are statistically different from the control (ANOVA) = $P < 0{\cdot}0005$.

3.3 *Growth*

Trends obtained for growth of the tested 5 groups are shown in figure 1. During the 75 days, the fish grew from 10 g to 36, 33, 30, 27 and 26 g in the group receiving 5, 2·5, 1, 10 and 0 mg ethylestrenol/kg fish. All the experimental groups receiving low dosages of ethylestrenol (5, 2·5 and 1) were significantly ($P < 0.001$) heavier than the control group (0 mg). The data clearly indicate that ethylestrenol is a growth promoter, when administered at about 5 mg/kg fish twice a week. Administration of 5 mg of ethylestrenol/kg fish is the optimal level in the fish *C. striatus*.

3.4 *Conversion efficiency*

Individuals in the control group converted the food at an average efficiency of 23 %. Ethylestrenol supplementation with diet promoted the efficiency to 39 % when supplemented at the optimum dose of 5 mg/kg fish. Conversion rate of the fish in the control group was around 48 J/g/day. Increasing the dosage 1, 2·5, 5 and 10 mg/kg enhanced the rate to 79, 78, 83 and 69 J/g/day.

4. Discussion

The present study has shown that ethylestrenol is not an appetite stimulant but an anabolic growth promoter of juvenile *C. striatus* and this observation confirms the

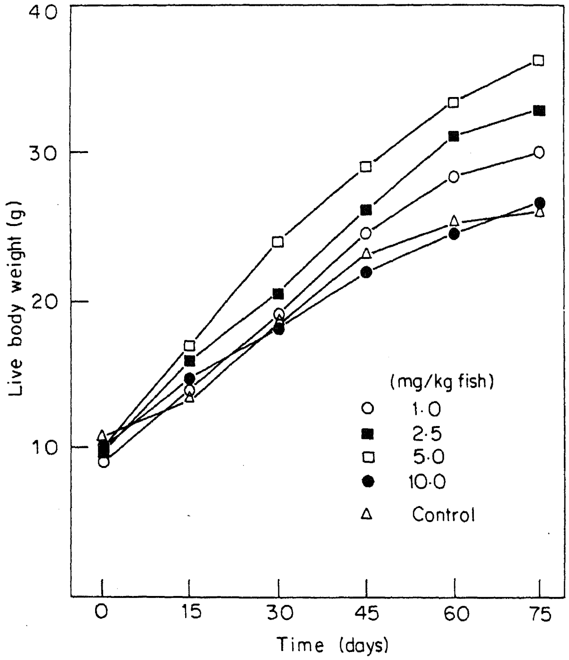

Figure 1. Effect of ethylestrenol supplemented with diet, on the growth of *C. striatus*. Each value represents the average performance of 3 individuals.

Table 2. Influence of steroid hormones on growth of commercially important fishes.

Species	Hormone	Optimum dose	Function of the hormone	Remarks	Reference
Oncorhynchus kisutch	17α-methyltestosterone	10 mg/kg diet	Growth promoter	Cr not estimated	McBride and Fagerlund (1973)
O. tshawytscha	17α-methyltestosterone	1 mg/kg diet	Growth promoter	Cr not estimated	McBride and Fagerlund (1973)
O. kisutch	17α-methyltestosterone	2·5 mg/kg diet	Growth promoter	Cr not estimated	Yu *et al* (1979)
	Testosterone	2·5 mg/kg diet	Growth promoter	Cr not estimated	Yu *et al* (1979)
	Estradiol	2·5 mg/kg diet	Growth promoter	Cr not estimated	Yu *et al* (1979)
O. kisutch	17α-methyltestosterone	1 mg/kg diet	Growth promoter	Cr not estimated	Fagerlund *et al* (1979)
Cyprinus carpio	17α-methyltestosterone	2·5 mg/kg diet	Growth promoter	Cr not estimated	Lone and Matty (1980)
	Testosterone	10 mg/kg diet	Growth promoter	Cr not estimated	Matty and Lone (1979)
	Adrenosterone	1 mg/kg diet	Growth promoter	Cr not estimated	Matty and Lone (1979)
	Ethylestrenol	2·5 mg/kg diet	Growth promoter	Cr not estimated	Lone and Matty (1983)
Channa striatus	17α-methyltestosterone	10 mg/kg fish	Growth promoter and appetite stimulant	Cr estimated	Nirmala and Pandian (1983)
	Testosterone	20 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Testosterone propionate	10 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Diethylstilbestrol	10 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Docabolin	30 mg/kg fish	Growth promoter and appetite stimulant	Cr estimated	Nirmala and Pandian (1983)
	Diethylstilbestrol dipropionate	30 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Estroid	30 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
Heteropneustes fossilis	17α-methyltestosterone	40 mg/kg fish	Growth promoter and appetite stimulant	Cr estimated	Sindhu and Pandian (1984)
Channa striatus	Ethylestrenol	33 mg/kg diet	Growth promoter	Cr estimated	Present study

Cr = Feeding rate

previous reports of Simpson (1976) and Lone and Matty (1983). Lone and Matty (1983) reported that a dose of 5 mg ethylestrenol/kg diet promotes maximum growth efficiency in juveniles of *Cyprinus carpio* without increasing feeding rate. Since Simpson (1976) and Lone and Matty (1983) fixed the dose of ethylestrenol with reference to unit weight of diet, there is a need to recalculate our data which are expressed in unit of dose per kilogram fish. Such calculation indicated that the optimum dose that maximises *C. striatus* growth is as high as 33 mg/kg diet. This high value may be compared with optimum doses reported for *C. carpio* (2·5 mg/kg diet: Lone and Matty 1983) and *Salmo gairdneri* (12·5 mg/kg diet: Simpson 1976). Clearly the anabolic property is realised in fishes, when ethylestrenol is supplemented but the optimum dose of ethylestrenol is a species specific character.

In *Salmo gairdneri* (Simpson 1976) and *C. carpio* (Lone and Matty 1983) the administration of optimum of ethylestrenol promoted the growth efficiency by about 17% higher than the control. An equal increase in efficiency was also observed in *C. striatus*. Irrespective of species specific variations in the efficiency, the optimum dose of ethylestrenol increased the growth efficiency by about 1·5 times over that of the control.

Available information on endocrine influence on somatic growth of commercially important fishes is presented in table 2. Pandian (1982) summarised the relevant information into 3 groups—(i) those which promote growth by acting as appetite stimulant (e.g. 17α-methyltestosterone, docabolin and estroid), (ii) those which are anabolic growth promoter (e.g. ethylestrenol, diethylstilbesterol and diethylstilbesterol dipropionate) and (iii) those which act as anabolic growth promoter in one species but stimulate appetite in another species; testosterone acts as an appetite stimulant in *Oncorhychus kisutch* (Yu *et al* 1979) but serves as an anabolic growth promotor in *C. striatus* (Nirmala and Pandian 1983). A large number of publications claim one or other steroid as anabolic; however these publications do not report feeding rates of the control and treated fishes. Hence it is difficult to know whether the same steroids indicated in table 2 acted as anabolic growth promoter or as an appetite stimulant. Based on the present observations, ethylestrenol served as an anabolic steroid in the young ones of *C. striatus*.

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Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker)

R BASKARAN and H MD MOHIDEEN
Fish Vision Unit, PG Department of Zoology, The New College, Madras 600014, India

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Abstract. In bright light-adapted (25 lux) retina of *Channa punctatus*, the pigment in the epithelial cell is fully migrated into the epithelial cell protuberances. In dim light-adapted retinae (6, 3 and 1 lux), the pigment concentration gradually decreases into the tips of the epithelial cell protuberances and its concentration gradually increases in the origin of the epithelial cell protuberances. In dark-adapted retina, the pigment is fully expanded into the epithelial cell. The pigment concentration is more in the vitread region. The cones remain contracted in light-adapted retinae of various light intensities. The rods are visible only in dim light-adapted (6, 3 and 1 lux) retinae and invisible in dark (0 lux). The mechanism of pigment migration in this fish is different from those of other fish.

Keywords. Retinomotor responses; retinal epithelial pigment layer; epithelial cell processes; visual cell layer; bright-, dim- and dark-adaptation.

1. Introduction

It is now well established that the retinae in fishes respond variously when exposed to different light intensities. For example, the retina in juvenile Atlantic Salmon is light-adapted at the intensities of 10^{-1} ft-C and above; semi-adapted at 10^{-2} ft-C, and dark-adapted at 10^{-3} ft-C or below (Ali 1961). Also, it took minimum time to reach the maximum state of adaptation, higher the light intensity and vice versa (Ali 1962) and as in the spotted mackerel, *Pneumatophorus tapeinocephalus* (Kaivamura 1979). Conclusions similar to the above were drawn also by Weidemann (1966) by studying cones in the guppy exposed to different wavelengths of equal energy. A threshold of such energies was suggested to explain the transition from scotopic to photopic vision, when the Retinal epithelial pigment (REP) starts to disperse (Ali and Crouzy 1968).

Furthermore, when dark-adapted *Lepomis cyanellus* are exposed to light (1·4 lux), cone myoids contract with a linear rate of $1{\cdot}5 \pm 0{\cdot}1$ μm/min. The myoid region of the cone contracts from as much as 120 μm (midnight dark-adapted) to 6 μm in the fully light-adapted state (Burnside *et al* 1982). But the inner segment of the rods in *Sarotherodon mossambicus*, increases in length from 12 μm in the dark to 41 μm in the light (O'Connor and Burnside 1981). In yet another response, the square mosaic pattern of retinal cones changes during dark adaptation into a row mosaic in the guppy, *Poecilia reticulata* (Kunz 1980).

In a state of nature *Channa punctatus*, a fresh water teleost, are exposed to different light intensities necessitating light adaptation for a successful establishment in a chosen environment. The present studies were therefore undertaken to understand the above behavioural pattern in *C. punctatus*.

2. Material and methods

C. punctatus obtained from fish market were kept in a glass tank supplied with aerated tap water. The fish were fed daily on a diet of fish food. The total length of the fish ranged from 15–18 cm.

2.1 *Experimental method*

A rectangular wooden box with 5 sides only, covered the glass tank in which the fish were kept. The wooden box had a small square opening through which a 60 watt bulb was focussed. Double cell colorimetric (Biochem) filters were used for producing various light intensities inside the tank. A lux meter was used for measuring the light intensities. The water temperature ranged between 18°–22°C during the period of the experiments.

In the first experiment (25 lux), light from 60 watt bulb was directly focussed on to the tank through the square opening. Then the light intensity over the surface of the water was measured by the lux meter. After 24 hr of light-adaptation decapitated head of a fish was preserved in Bouin's fixative for 24 hr.

In 2nd (6 lux), 3rd (3 lux), 4th (1 lux) experiments red, green and blue filters of double cell colorimeter (Biochem) was inserted into the square opening of the wooden box and the 5th dark-adaptation experiment was conducted in a light proof room. The procedures adopted for these experiments were identical with those of the first experiment.

2.2 *Histology*

After fixation of eyes in Bouin's fixative, the retinae were embedded in paraffin. The blocks were cut at 3 μ, stained with Harris Haemotoxylin and counterstained with alcoholic eosin. The sections were mounted in Distrene 80 dibutylphthalate xylene (DPX) and examined under the microscope.

2.3 *Measurements*

The thickness of the retina, retinal epithelial pigment layer (REPL) and visual cell layer (VCL) were measured with calibrated ocular micrometer. Two measurements of the REPL and VCL in small retinal sections and 3 measurements in large retinal sections, totally 150 measurements were taken from 5 experiments i.e., at the rate of 30 measurements for each experiment.

3. Results

In normal light, the cones are contracted, the rods and the pigments are expanded; but in dark, the cones are expanded, the rods and the pigments are contracted (Ali 1961). The contraction and expansion of cones and rods in *C. punctatus* are similar to those of other fish. But the pigment migration is different in this fish.

3.1 *Bright light-adaptation in* 25 *lux* (*figures* 1*A and* 2; *table* 1)

The pigment in the epithelial cells is fully migrated into the tips of Epithelial cell processes (ECP). Consequently REPL appears transparent in that region (18 μ). As a result of contraction of the cones, the ellipsoids of the cones lie close to the external limiting membrane with VCL measuring (16 μ). The rods cannot be identified because they are masked by pigment in the ECP.

3.2 *Dim light-adaptation in* 6, 3 *and* 1 *lux* (*figures* 1*B–D and* 2; *table* 1)

When the light intensity is decreased, the pigment concentration gradually decreases in the vitread regions of the epithelial cells and gradually increases in the sclerad regions of the epithelial cells. The transparency of REPL also decreases, when the light intensity is decreased. This clearly indicates the migration of pigment from ECP to REPL. The cones remain in a contracted state. The rods are faintly visible in the masking pigment of ECP (6, 3, 1 lux).

3.3 *Dark-adaptation in* 0 *lux* (*figures* 1*E and* 2; *table* 1)

The pigment is fully dispersed in the epithelial cells. The pigment concentration is also high in the tips of the ECP. This indicates the reversal dispersion of pigment into the tips of the ECP from REPL. The rods and cones could not be identified in the retina, because they are completely masked by the ECP.

4. Discussion

In bright light (25 lux) in both the present studies on *C. punctatus* and investigations on goldfish and bullhead (Ali 1964a), the pigment is fully migrated into the vitread regions of the ECP (figures 1A and 2). But, *C. punctatus* differs from the goldfish and bullhead in one respect namely while the sclerad regions of the REPL is fully transparent in *C. punctatus*, some amount of pigmentation is still present in those of goldfish and bullhead. Nevertheless, the expansion of pigment in *C. punctatus* is similar to that observed in various species of salmon (Ali 1959, 1971, 1975); in bullhead and goldfish (Ali 1964a); in herring (Blaxter and Jones 1967); and in toadfishes (Nicol 1980).

In dim light-adaptation (6, 3 and 1 lux) both in the present studies on *C. punctatus* and salmon (Ali 1959, 1971, 1975); bullhead and goldfish (Ali 1964a); and herring (Blaxter and Jones 1967) migration of pigment takes place from the ECP to REPL (figures 1B–D and 2). While in the fishes cited the pigment concentration gradually decreases with decreasing light intensity; in *C. punctatus* it gradually increases with decreasing light intensity.

In dark-adapted retina of *C. punctatus*, the pigment migration is first into the REPL and from the REPL to ECP (figures 1E and 2), which is contrary to that observed in salmon (Ali 1959, 1971, 1975); bullhead and goldfish (Ali 1964a); herring (Blaxter and Jones 1967); and snook (Eckelbager *et al* 1980). But in the bullhead pigment concentration is more in the sclerad regions of the epithelial cells. In *C. punctatus* the

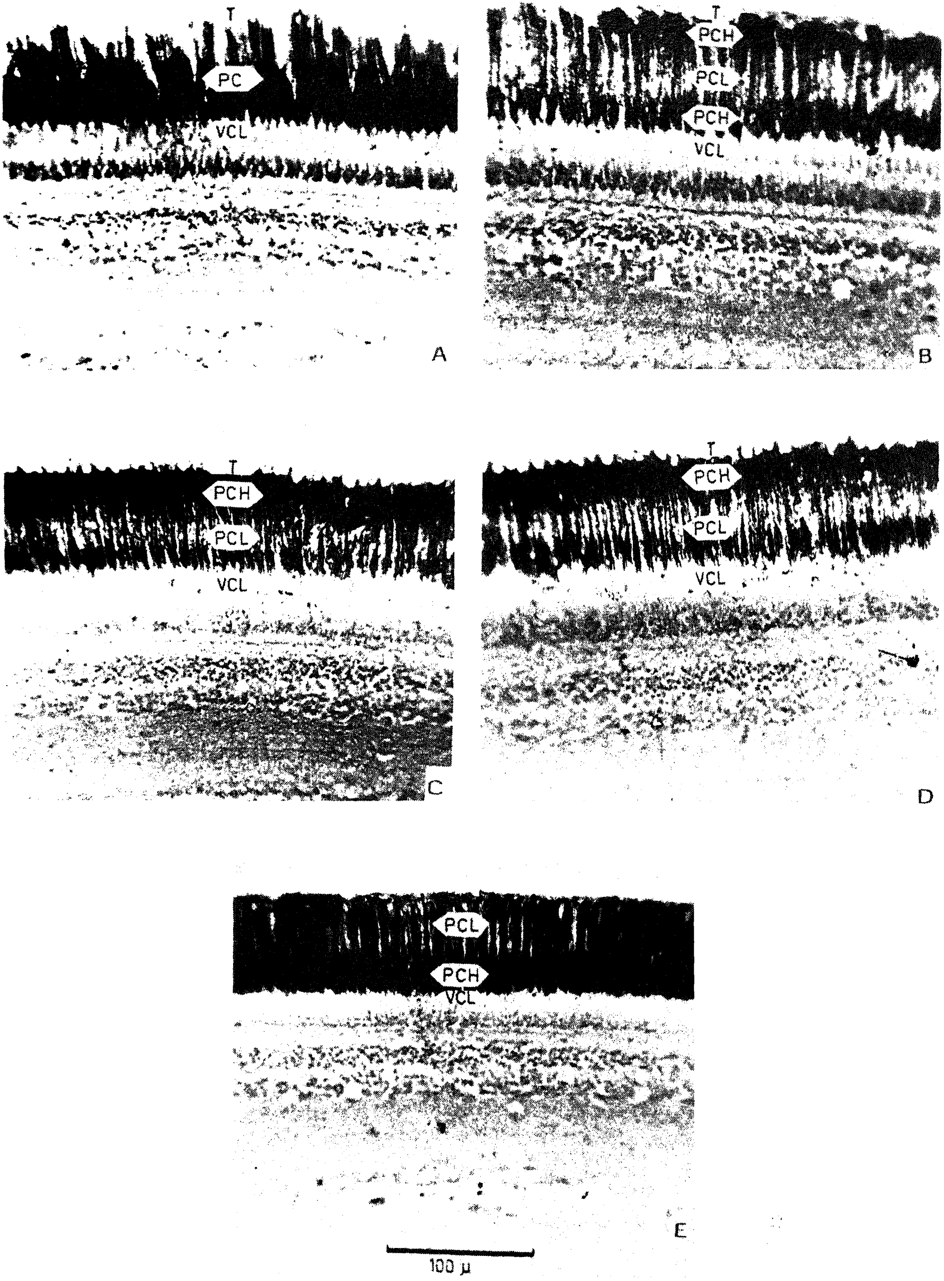

Figure 1. **A–D.** Light-adapted retina of *C. punctatus* (A, 25 lux; B, 6 lux; C, 3 lux; D, 1 lux). **E.** Dark-adapted (0 lux) retina of *C. punctatus*.

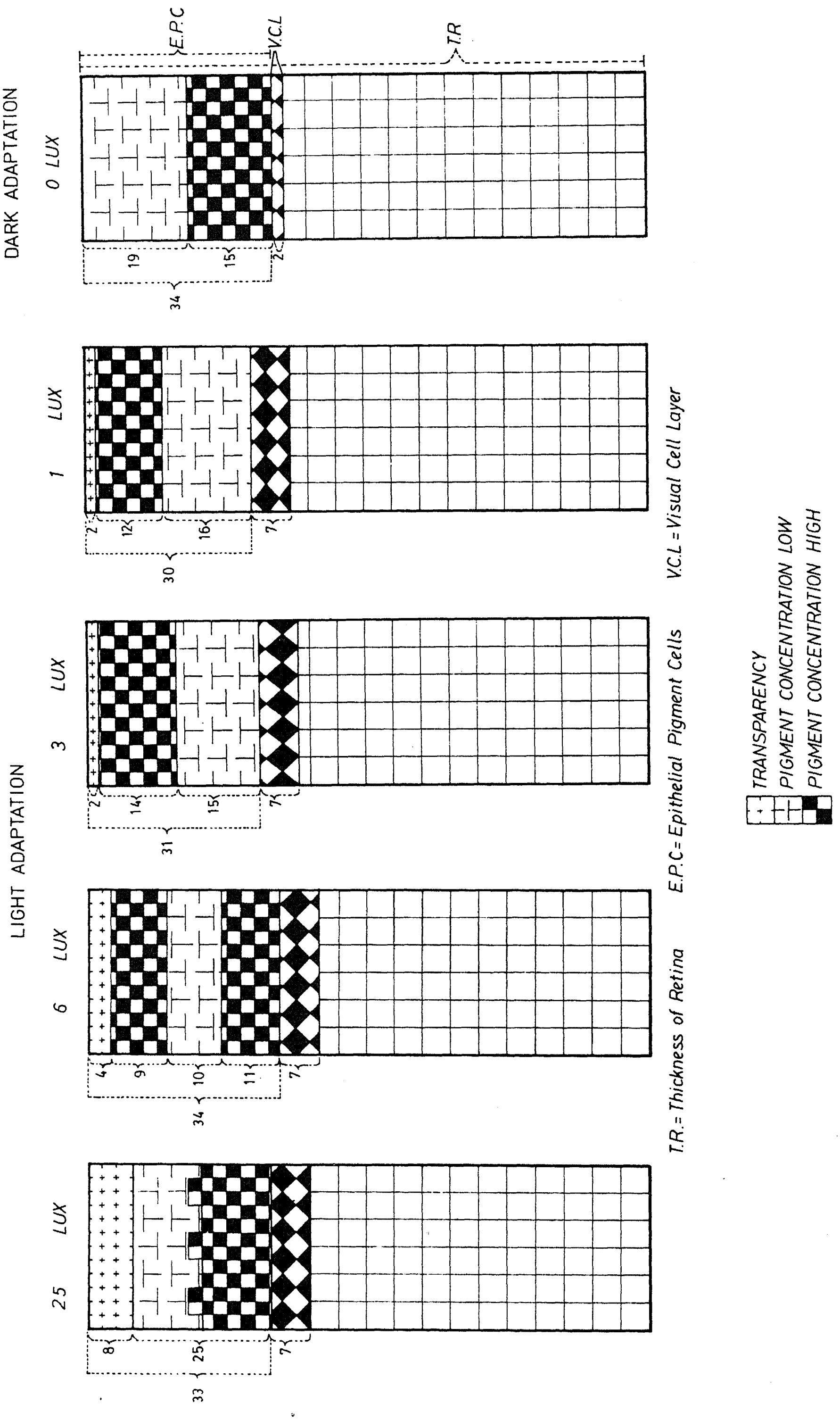

Figure 2. Histogram of the concentration of pigment in retinal epithelial pigment cells and thickness of visual cell layer.

Table 1. Thickness of retinal epithelial pigment and visual cell layer.

				PL				
	TR	VCL	PL	PC	PCH	PCL	PCH	T
Light adaptation in 25 lux								
μ	228	16	75	57				18
%	100	7	33	25				8
Light adaptation in 6 lux								
μ	226	15	76		25	22	19	10
%	100	7	34		11	10	9	4
Light adaptation in 3 lux								
μ	234	16	72			36	31	5
%	100	7	31			15	14	2
Light adaptation in 1 lux								
μ	232	15	70			37	28	5
%	100	7	30			16	12	2
Dark adaptation								
μ	176	3	66		28	38		
%	100	2	34		15	19		

(PC, Pigment concentration; PCH, pigment concentration high; PCL, pigment concentration low; PL, pigment layer; T, transparency; TR, thickness of retina; VCL, visual cell layer).

opposite result is observed. The present studies are insufficient to throw light on the mechanism leading to the observed behavioural response and adaptation in *C. punctatus*.

The cones of *C. punctatus* remain in contracted state (figures 1A–D and 2) when the fish is subjected to various light intensities. The present studies do not, however indicate whether or not the cones are in the same state in dark also, since they are completely masked by REP (figures 1E and 2). That the cones did not show any retinomotor responses both in dark and light has been reported in bream (Garten 1907); in walleye and sauger (Ali and Anctil 1977); in goldeye and mooneye (Wagner and Ali 1978); in bigeyes (Wang *et al* 1980); in *Oreocromis mossambicus* (Jalaluddin Sherieff 1985) and in several other species (Ali and Wagner 1975).

The rods of *C. punctatus* are expanded in bright light (25 lux), and are in a contracted state in dim light (6, 3 and 1 lux) and in dark (0 lux). But the rods are visible only in dim light-adapted (6, 3 and 1 lux) retinae (figures 1B–D) and could not be identified both in bright light (25 lux) and darkness (figures 1A and 1E); whereas the rods are visible in the dark-adapted retina of other fish (Ali 1959, 1964a, 1971, 1975; Blaxter and Jones 1967).

A question that arises from the above results is as to how does the pigment function in light and dark?

In bright light and dim light-adapted retinae of *C. punctatus* the position of rods and cones and the direction of pigment migration are more or less identical to that of other species (Garten 1907; Ali 1959, 1964a, 1971, 1975; Blaxter and Jones 1967). So, the mechanism of transmission of light may be identical to that of other species described above.

In dark-adapted retina of *C. punctatus* the pigment is expanded in the epithelial cells (figure 1E). Chiarini (1904) considered that the function of the dispersed REP was nutritive and that it compensated for the loses by rods and cones. So, the expansion of pigment in dark-adapted *C. punctatus* may be for the purpose of nutrition. It is possible that in dark, a small amount of light may enter the rods through the gap of the ECP below, and since the cones also serve as light funnels (Ali and Wagner 1976) enables *C. punctatus* to see the object in dark.

Why does the pigment expand in dark? Ali (1964b) in his unilateral illumination experiment with the goldfish and Bimes *et al* (1966) on *Cyprinus carpio* stated that the REP is to a greater extent under hormonal control than the visual cells which are the first order neurons. From the present investigations it is believed that the different types of migration of pigment observed in *C. punctatus*, may be under the influence of a special hormone.

5. Conclusion

1. In *C. punctatus* the cones remain contracted in various light intensities and the rods are not visible in dark.

2. The pigment concentration in the retina under various light intensities clearly indicates that the pigment migrates first from REPL to the tips of the ECP in bright light (25 lux) and then from the tips of the ECP towards the direction of REPL in dim light (6, 3 and 1 lux). In dark, the pigment migration from ECP to REPL is completed and then it migrates from REPL to the tips of the ECP.

3. In this fish, the thickness of pigment concentration in epithelial cells gradually increases when the light intensity is decreased.

4. The transparency of REPL gradually decreases when the light intensity is decreased.

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Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton)*

M PEER MOHAMED and M N KUTTY†

Central Inland Fisheries Research Institute, Riverine and Lacustrine Division, 24 Pannalal Road, Allahabad 211 002, India

†FAO African Regional Aquaculture Centre, PMB 6165, Port Harcourt, Nigeria

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Abstract. Oxygen consumption and carbon dioxide production increased with increase in random (spontaneous) swimming activity in mullet, *Rhinomugil corsula* in high oxygen (normoxia), but the oxygen consumption in low oxygen (hypoxia) was negatively correlated with swimming activity in tests at both 30 and 35°C. The RQ of mullet under hypoxia always remained over unity and increased with increase in activity, clearly indicating intense utilization of anaerobic energy. The RQ under normoxia remained near unity irrespective of random swimming activity change. The AQ of normoxic and hypoxic mullet showed opposing trends, the quotient increasing with decreasing activity in the former and increasing with activity increase in the latter. The AQ change suggests increased protein utilization in quieter fish when adequate ambient oxygen is available, but the hypoxic mullet utilizes more protein the more active it is. The latter change in AQ is consonant with the change in hypoxic RQ and it is likely that increased anaerobic energy utilization demanded by the increased activity is accompanied by increased protein breakdown and ammonia release, thereby helping in acid-base balance and ionic (Na^+) regulation. Results obtained at 30 and 35°C are almost identical. Within the small range temperature does not seem to cause a marked metabolic difference in mullet.

Keywords. Ambient oxygen; normoxia; hypoxia; respiratory quotient; ammonia quotient; swimming activity; *Rhinomugil corsula*.

1. Introduction

The metabolic quotients, RQ and AQ‡ in fish have been used as indices of anaerobic metabolism (Kutty 1968, 1972; Kutty *et al* 1971a; Peer Mohamed and Kutty 1981; Peer Mohamed 1982; Peer Mohamed and Kutty 1983a, b) and protein utilization (Stroganov 1956; Kutty 1972; Kutty and Peer Mohamed 1975; Sukumaran and Kutty 1977; Peer Mohamed and Kutty 1981, 1983a, b), respectively. While there are several studies on the influence of random (spontaneous) activity on oxygen consumption in fish (Spoor 1946; Beamish and Mookherjii 1964; Beamish 1964; Smit 1965; Peer Mohamed *et al* 1978; Peer Mohamed 1982; Peer Mohamed and Gupta 1984) the influence of random activity on RQ has been studied only in a few cases (Kutty 1968; Kutty and Peer Mohamed 1975; Peer Mohamed and Kutty 1981, 1983a, b; Peer Mohamed 1982).

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‡RQ and AQ denote Respiratory Quotient and Ammonia Quotient which are mole to mole relations of carbon dioxide produced to oxygen consumed, and ammonia excreted to oxygen consumed, respectively.

Under aerobic conditions random activity did not appear to have any effect on the RQ of goldfish and rainbow trout (Kutty 1968), but the AQ might change with random activity (Kutty and Peer Mohamed 1975; Peer Mohamed 1982). Intense forced activity has been shown to influence both RQ and AQ in fishes (Kutty 1968, 1972; Sukumaran and Kutty 1977). Also it is not known how random activity and ambient oxygen would together influence anaerobic energy utilization and protein degradation. A study of RQ and AQ in mullet under random activity under various oxygen conditions would throw light in this regard. In the present study simultaneous measurements of oxygen consumption, carbon dioxide output, ammonia excretion and random swimming activity have been made in the mullet, *Rhinomugil corsula* at various ambient oxygen concentrations below air saturation at two temperatures, 30 and 35°C. Since most of the nitrogen excreted by the freshwater fish is in the form of ammonia (Black 1957) as shown in the present material (*Rhinomugil corsula*) as well (Sukumaran and Kutty 1977) it is felt that a reasonable pattern of protein degradation can be obtained from measurements of ammonia excreted.

In freshwater fish under anaerobiosis, ammonia produced may be of use in combating acidosis and also in conservation of Na^+ (Prosser and Brown 1961; Garcia Romeu and Motais 1966; Forster and Goldstein 1969; Kutty 1972; Peer Mohamed and Kutty 1981). In this context as well, the present study on changes in RQ and AQ in mullet especially under hypoxia will be of interest.

2. Material and methods

Rhinomugil corsula (Hamilton), chosen for this study, is a fresh-water mullet abundant naturally in Gangetic and Mahanadhi river systems in North India; the fish was transplanted to Vaigai Dam by The Tamil Nadu Department of Fisheries (Kutty *et al* 1971b), and now the fish are breeding and thriving in freshwater in the Vaigai reservoir (Narayanan 1974). *R. corsula* collected from Vaigai Dam, were acclimated to freshwater at 30 ± 1°C and 35 ± 1°C for atleast 15 days before experiment, and tested under acclimation temperature. The fish were deprived of food for 36 hr (Fromm 1963; Beamish 1964; Kutty 1972; Peer Mohamed and Kutty 1981) before experiments.

Thirty-two mullet were used in these tests; they ranged in weight from 33·9–53·1 g (mean 41·2 g) and total length from 16·7–19·3 cm (mean 17·4 cm).

2.1 *Apparatus*

A modification of Fry's respirometer (Kutty *et al* 1971b) was used for the present experiments. Mainly it consisted of two units, an electronic counter and a transparent 'Perspex' annular respirometer having a capacity of 3 litres. When the fish moved it cut the light beams focussed on photocells in the centre of the annulus and the event was recorded by the electronic counter (more details of the apparatus are given in Kutty *et al* 1971b).

2.2 *Fish medium and recirculation*

The natural well-water available was very hard and it was unsuitable as fish medium for accurate determination of carbon dioxide production of fish. Therefore, as explained in

Kutty *et al* (1971a) decarbonated water of controlled pH (Bosworth *et al* 1936; Kutty 1968) was prepared and used as fish medium. The medium was pumped from a ground level reservoir (70 L) to an overhead reservoir of the same size. The latter was provided with a thermostat and a constant level device (Electric Control Equipment Co., Madurai) which activated the pump. Water from the overhead reservoir flowed through the respirometer to the ground level reservoir from which it was recirculated.

2.3 *Experimental procedure*

The experimental fish was transferred to the respirometer and the fish medium at the acclimation temperature, was circulated through the respirometer overnight. At the start of the experiment, initial water samples of run I were collected and circulation of water through the respirometer was cut off. After an interval of 60 min final samples were collected. At each sampling (initial and final of each run), 3 separate water samples were collected for analyses of dissolved oxygen, total carbon dioxide and ammonia. The size of each sample was 30 ml for dissolved oxygen, 60 ml for total carbon dioxide and 60 ml for ammonia (25 ml collected first as rinse was discarded). The figure in the activity counter was recorded immediately after each sampling. After taking the final samples of run I the respirometer was not opened to the circulating water for complete flushing but approximately 175 ml of fish-medium was circulated once or twice through the respirometer for mixing it with the water remaining unflushed in the respirometer. Then the initial samples of next run (run II) (as described above) were collected. The overall time for sampling and adding water was about 3 min. During the last run, the final samples were collected only after the fish reached the asphyxial oxygen level, as indicated by the beginning of equilibrium loss of the fish. Then the respirometer was flushed with air-saturated decarbonated water to revive the animal.

Data from the test runs were categorised into 2 groups, depending on the mean ambient oxygen concentration: (i) those with ambient oxygen values below air saturation but above 50 % air saturation (referred here as 'normoxia') and (ii) those with oxygen values below 50 % air saturation down to asphyxial oxygen (referred here as 'hypoxia'). Subsequent analyses of data are done accordingly.

2.4 *Methods of water analyses*

2.4.1 *Dissolved oxygen:* The unmodified Winkler method was followed (American Public Health Association 1965). The size of the sample used for titration was 25 ml.

2.4.2 *Total carbon dioxide:* Direct measurement of total carbon dioxide by distillation and subsequent titration was made by the method described by Kutty *et al* (1971a), which is a modification of Maros-Schulek technique (Maros *et al* 1961).

2.4.3 *Ammonia:* The method followed was described by Kutty (1972) in which some modifications were incorporated as suggested by Stroganov (1962), which was in general agreement with that given in Standard Methods (American Public Health Association 1965). Fifty ml of water samples were distilled, the distillate nesslerised, and the optical density read in a Bausch and Lomb spectrophotometer (Spectronic-20) at a wavelength of 420 μ. Ammonia free water (American Public Health Association 1965) was always used for blank and to prepare the reagents.

3. Results

Data on oxygen consumption, carbon dioxide production, RQ and AQ in relation to random swimming activity in mullet *R. corsula* exposed to oxygen concentration near air saturation (normoxia) and below 50% air saturation down to asphyxial oxygen concentration (0·8 mg l^{-1}) (hypoxia), are plotted in semilogarithmic graphs and are displayed in figure 1 (30°C) and 2 (35°C). The regression equations concerned are presented in table 1. The data on ammonia excretion are not indicated in figures 1 and 2

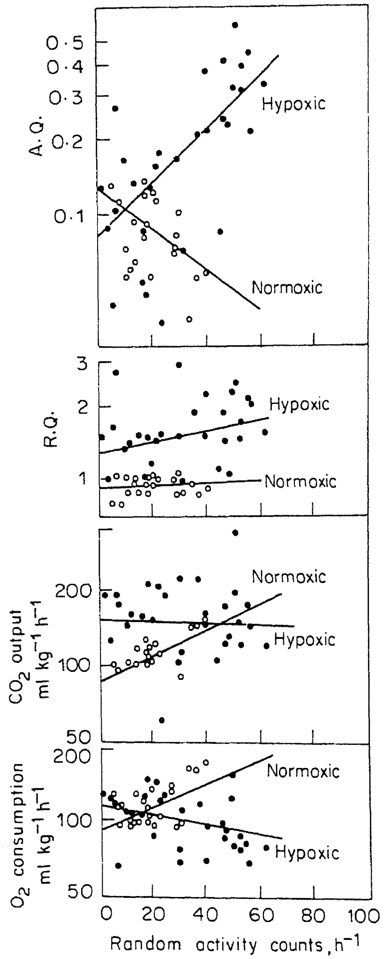

Figure 1. Oxygen consumption, carbon dioxide production, RQ and AQ in relation to random swimming activity in mullet, ***Rhinomugil corsula*** (acclimation and test at 30°C) exposed to oxygen concentration near air saturation (normoxia) and to oxygen concentration below 50% air saturation down to asphyxial oxygen concentration (0·8 mg l^{-1}) (hypoxia). Data for normoxia and hypoxia are indicated by open and closed symbols respectively. Regression lines are fitted to plots in the figure by the method of least squares.

Table 1. Regression equations of logarithms of O_2 consumption, CO_2 production, NH_3 excretion (ml kg^{-1} hr^{-1} in each case), RQ and AQ (Y) on random swimming activity (counts hr^{-1}) (X) in the mullet, *Rhinomugil corsula*, under 'hypoxia' (ambient O_2 below 50% air saturation down to asphyxial oxygen concentration) and 'normoxia' (ambient O_2 near air saturation) at 30°C and 35°C.

	30°C	35°C
	Hypoxia	
O_2	: $\log Y = 2{\cdot}07044 - 0{\cdot}00245\ X$	$\log Y = 2{\cdot}00307 - 0{\cdot}00036\ X$
CO_2	: $\log Y = 2{\cdot}17358 - 0{\cdot}00031\ X$	$\log Y = 2{\cdot}14488 + 0{\cdot}00057\ X$
NH_3	: $\log Y = 0{\cdot}92250 + 0{\cdot}01000\ X$	$\log Y = 0{\cdot}83530 + 0{\cdot}00820\ X$
RQ	: $\log Y = 0{\cdot}10799 + 0{\cdot}00197\ X$	$\log Y = 0{\cdot}12856 + 0{\cdot}00361\ X$
AQ	: $\log Y = -1{\cdot}06161 + 0{\cdot}01056\ X$	$\log Y = -1{\cdot}01085 + 0{\cdot}00361\ X$
	Normoxia	
O_2	: $\log Y = 1{\cdot}96761 + 0{\cdot}00503\ X$	$\log Y = 2{\cdot}04390 + 0{\cdot}00125\ X$
CO_2	: $\log Y = 1{\cdot}93430 + 0{\cdot}00446\ X$	$\log Y = 1{\cdot}99140 + 0{\cdot}00292\ X$
NH_3	: $\log Y = 0{\cdot}95080 + 0{\cdot}00250\ X$	$\log Y = 1{\cdot}03220 + 0{\cdot}00110\ X$
RQ	: $\log Y = -0{\cdot}03010 - 0{\cdot}00068\ X$	$\log Y = -0{\cdot}03720 - 0{\cdot}00056\ X$
AQ	: $\log Y = -0{\cdot}89420 - 0{\cdot}00898\ X$	$\log Y = -1{\cdot}01080 - 0{\cdot}00020\ X$

to avoid crowding in the figures, but their extent can be judged from a comparison of AQ and corresponding values of oxygen consumption.

It is interesting to check if the changes in the metabolic rates and quotients can be correlated with changes in random swimming activity at different ambient oxygen concentrations. Extrapolation of a line drawn through such plots of metabolic rates to 'zero' activity can be used for estimating the standard metabolism (Spoor 1946; Smit 1965; Kutty 1968; Kutty *et al* 1971b; Peer Mohamed *et al* 1978; Peer Mohamed and Gupta 1984). For such estimations, usually data obtained from simultaneous measurements of metabolism and activity of a single fish are used for plots. In the present study, the data available for individual fish are too small for such treatment. Therefore data for all individuals of mullet tested under similar oxygen concentrations are pooled. This we believe is permissible in view of the fact that the fish used were almost of similar size and some useful information can thus be made out with the present results. The trends of metabolic rates and quotients are essentially same in mullet at 30 and 35°C (figures 1 and 2). So there is some uniformity of results obtained, in spite of pooling of data from different fishes.

The standard (basal) values of metabolic rates and quotients of normoxic and hypoxic *R. corsula* at 30 and 35°C are given in table 2 as estimated from the regression equations in table 1. It is seen that the standard values at 30 and 35°C are remarkably close to each other, suggesting that temperature effect between the test temperatures is minor, probably because these temperatures are close to each other and are within the upper range to which the mullet is normally exposed during a good portion of the year. In general this is true of the other routine metabolic rates presented as well.

4. Discussion

Slopes of regression lines of oxygen consumption and carbon dioxide production in mullet under adequate oxygen (normoxia) (figure 1) indicate that both rates increase

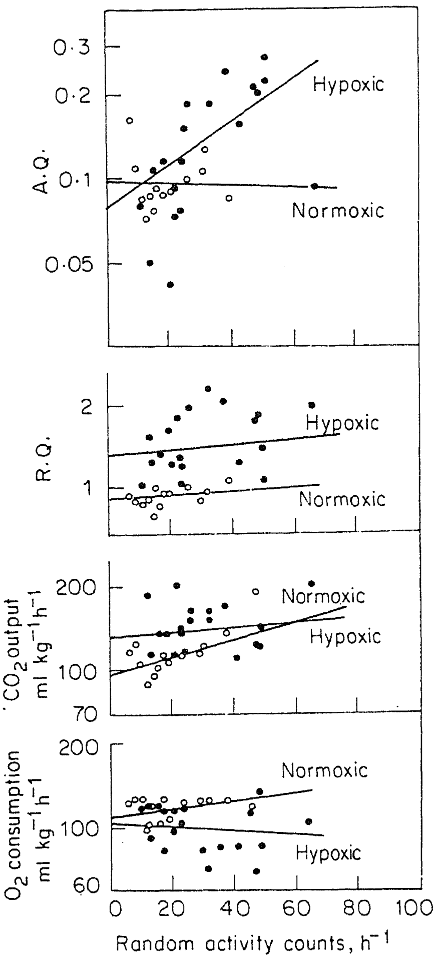

Figure 2. Oxygen consumption, carbon dioxide production, RQ and AQ in relation to random swimming activity in mullet, *Rhinomugil corsula* acclimated to and tested at 35°C. For further explanation see legend to figure 1.

Table 2. Estimated standard metabolic rates and quotients of mullet, *Rhinomugil corsula* acclimated to and tested at 30 and 35°C under high (normoxia) and low (hypoxia) oxygen.

Metabolic rate/quotient (ml kg^{-1} hr^{-1})	Normoxia		Hypoxia	
	30°C	35°C	30°C	35°C
Standard O_2 consumption	93	111	118	101
Standard CO_2 production	86	98	149	140
Standard NH_3 excretion	9	11	8	7
Standard RQ	0·93	0·92	1·28	1·34
Standard AQ	0·13	0·10	0·09	0·10

with increase in random swimming activity. The fish is aerobic, maintaining an RQ close to unity (the change in RQ with random activity is negligible). In normoxic fish the AQ seems to increase with decrease in random activity, especially clear in the plot of values at 30°C (figure 1) suggesting a greater involvement of protein degradation, the quieter the fish, the proportionately higher its protein use as was also observed in *Tilapia mossambica* (Peer Mohamed 1982). This observation is in variance with the results of study on the influence of forced activity on AQ. In the latter case the AQ increased with increase in activity (Kutty 1972; Karuppannan 1972; Sukumaran and Kutty 1977). In *T. mossambica* it appeared that initial AQ (first hour of 5 hr exercise) at lower swimming velocity was less than the routine AQ (Kutty 1972; Karuppannan 1972). And it was suggested that in this case there might be a protective action of carbohydrates on protein. This might be the reason why the steady AQ (after 2–3 hr swimming) is correlated better with the initial RQ of fish under long-term exercise (Kutty 1972; Karuppannan 1972). It is not impossible that random activity (spontaneous random movement) and forced activity (intense exercise) have different relations to protein degradation and utilization, as indicated by NH_3 excretion. A quieter fish would utilize proteins more, but increase in random activity relatively more energy is required by the fish for several breaks and starts in swimming overcoming inertia each time (Brett 1964; Smit 1965; Kutty 1969). In this case carbohydrate utilization could be higher as long as adequate oxygen is available, thereby indicating lower AQ at higher random activity, more or less similar to the low initial AQ during the beginning of intense activity (continued exercise). In both these cases, fish under high random activity and initial phase of intense swimming (forced activity), the protective action of carbohydrates on proteins may be operative (Phillips 1970) and low AQs may result. In continued exercise a better correlation of AQ is always with the duration of exercise, protein utilization being high the longer the exercise, rather than with the increase in the intensity of exercise eventhough the latter does exist in apparent contrast with the AQ decrease with increase in random activity.

The trends of hypoxic metabolism with activity are quite different. The oxygen consumption of hypoxic mullet decreased with increase in random activity, and its RQ and AQ showed marked increase with increase in activity, in marked contrast with the trends of these parameters in normoxic mullet.

The level of carbon dioxide production in the hypoxic mullet is generally higher than the oxygen consumption, as indicated by the RQ plot. The anaerobic metabolism (RQ over unity) increased with increase in activity. The opposing trends of oxygen consumption and RQ in relation to random activity again suggest a compensatory increase in anaerobic energy utilization.

The negative slope of the oxygen consumption–activity relation of the hypoxic mullet can perhaps be explained as due to acid loading, or acidosis and subsequent reduction in oxygen capacity of the blood. Karuppannan (1972) also found that at high temperatures the relation of oxygen consumption and level of forced activity in *T. mossambica* may have a negative slope. There is evidence that the anaerobic metabolism was higher at higher activities in the hypoxic mullet, and therefore acid accumulation could also have been higher. The oxidative energy lack is compensated by increased protein degradation and anaerobic energy utilization. As has been suggested earlier herein, the coupling of increased protein degradation and anaerobic energy utilization may have significance in combating acidosis and also in conserving Na^+ during hypoxia, which may be specially required due to hypoxic stress on ion-osmotic mechanism.

From the scatters of hypoxic AQ values (figures 1 and 2) it is seen that the higher values were obtained in mullet at 30°C, some values going as high as 0·5. While it appears that much of the ammonia excreted by mullet under hypoxia was produced anaerobically, it is suggested that only values higher than a theoretically estimated maximum for anaerobic conditions can be confirmed as anaerobically involved (Sukumaran and Kutty 1977). In that context clearly anaerobic AQs (above 0·33) seem to occur in hypoxic mullet at 30°C only, but it is likely that under hypoxic conditions even the lower AQs are partially accounted for by anaerobically produced ammonia.

It is also interesting to compare the standard metabolism of the hypoxic and normoxic mullet. Because of the opposing trends in the slopes of the regression lines of oxygen consumption and random activity at both 30 and 35°C, the standard metabolism under normal and hypoxic conditions is not markedly different, but the standard carbon dioxide production and RQ are different, the latter being always higher in the hypoxic mullet, suggesting that anaerobic metabolism, can be intense in quieter fish as well under hypoxic conditions.

5. Conclusion

There does not appear to be any marked difference between the metabolic rates of mullet at 30 and 35°C, eventhough the RQ and AQ at the temperature range is perhaps close and is probably near a flattened optimum level. While further statistical treatment of the limited data available appears unwarranted, as explained, the general similarity of trends of metabolic rates and quotients in relation to random swimming activity in hypoxic and normoxic mullet, at 30 and 35°C, appears to confirm the validity of the present observations. There are no other similar observations on fish, as far as we are aware, and within the general framework of the present study the results are valid and clearly point out the need for further study in the area of organismal physiology. Taking this approach the ecophysiologist or the autecologist would perhaps succeed in unravelling some of the unanswered questions in bioenergetics especially with reference to the coupling of anaerobic energy utilization and enhanced protein metabolism as it is related to the activity of the whole animal.

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Distribution and abundance of prawns in the freshwater habitats of Bangalore, South India

K V ANANTHA RAMAN, S RAVICHANDRA REDDY and KATRE SHAKUNTALA

Department of Zoology, Bangalore University, Jnana Bharathi, Bangalore 560 056, India

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Abstract. Fifty three freshwater habitats located in and around Bangalore (South India) were surveyed for studying the distribution and abundance of prawns. The survey indicated that, the habitats were rich in prawn fauna, harbouring one species of Palaemonid, *Macrobrachium lanchesteri* (de Man) and 3 species of Atyids, *Caridina nilotica* (Roux) var *bengalensis*, *Caridina rajadhari* (Bouvier) and *Caridina weberi* var *sumatrensis* (de Man). *Macrobrachium lanchesteri* and *Caridina nilotica* were more widely distributed than *Caridina rajadhari* and *Caridina weberi*. The results throw light on the pattern of distribution and species composition of these prawns in relation to the nature of the habitats.

Keywords. Prawns; distribution; abundance.

1. Introduction

In an earlier report, Anantha Raman *et al* (1978) have described the occurrence of one species of palaemonid and 3 species of Atyid prawns in the freshwater habitats of Bangalore. All these prawns are either important commercially (Johnson 1968; Guerrero and Guerrero 1979) or have ecological significance (Hart 1980) in freshwater habitats. The present paper describes the distribution of these prawns in the lentic and lotic habitats of Bangalore.

2. Materials and methods

During the early part of 1978, 53 freshwater habitats located in and around the North and South taluks of Bangalore (figure 1) were surveyed for the occurrence and distribution of freshwater prawns. The nature of each habitat was noted. From each habitat, prawns were collected using a plough net (mesh size: 0·75 × 0·75 cms), specially designed for the purpose. This facilitated the capture of prawns above the size of 10 mm in total length and ensured that all available species were represented. In each habitat, the net was operated randomly 5 times for a total distance of 20 m and using the dimensions of the net and the distance ploughed, the average number of prawns collected per 20 m^2 was determined. After collection, the prawns were preserved in ice and transported to the laboratory. In the laboratory, the samples of prawns collected were identified using standard keys (Tiwari 1947; Holthuis 1950, 1965, 1978) and the distribution patterns of these prawns in the different habitats were recorded.

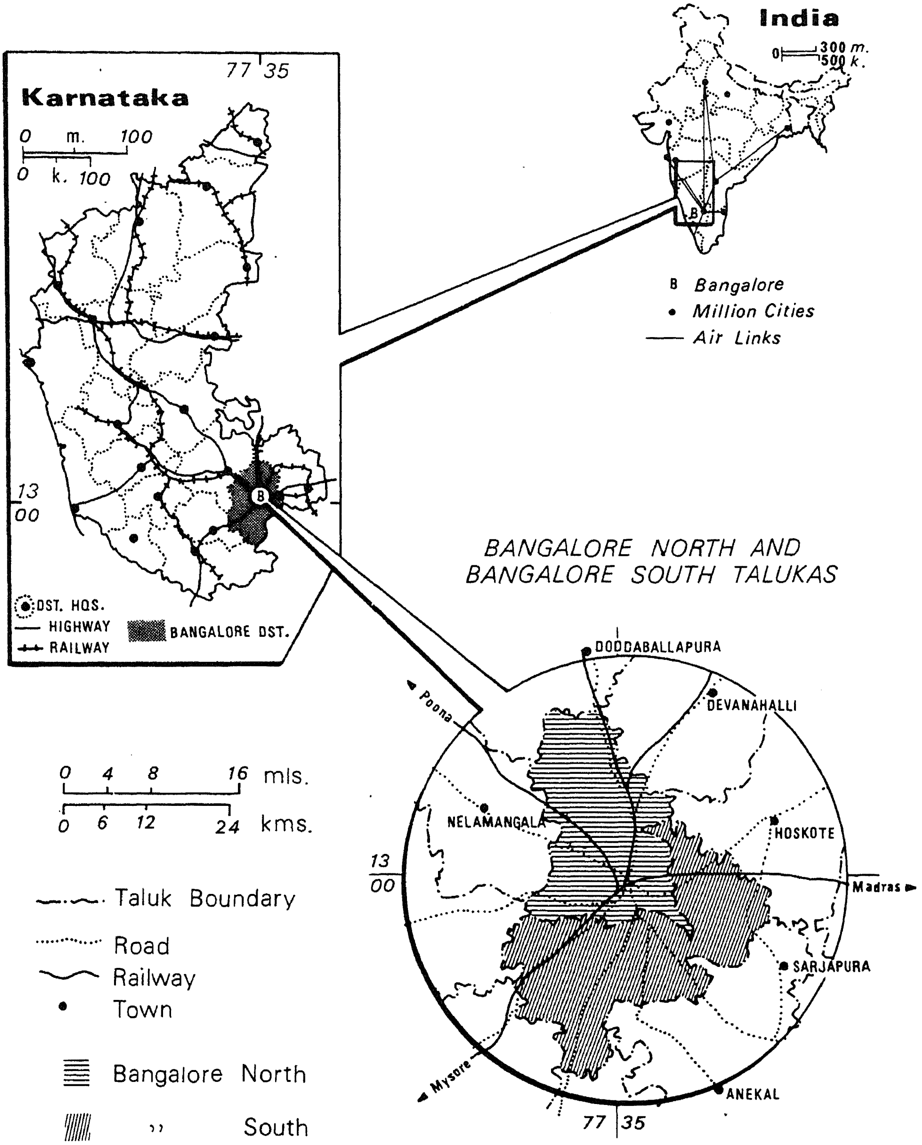

Figure 1. Topographic location of taluks of Bangalore District.

3. Observations and results

Except for one lotic habitat represented by the Vrishabhavathi rivulet, the inland habitats in and around Bangalore are mostly represented by perennial or seasonal lentic habitats which include pools/ponds/tanks/reservoirs/lakes. Most of the habitats are rainfed and the water is regularly used for purposes of irrigation. In many of the habitats, human activities have been considerable, leading to the drainage of domestic/industrial wastes.

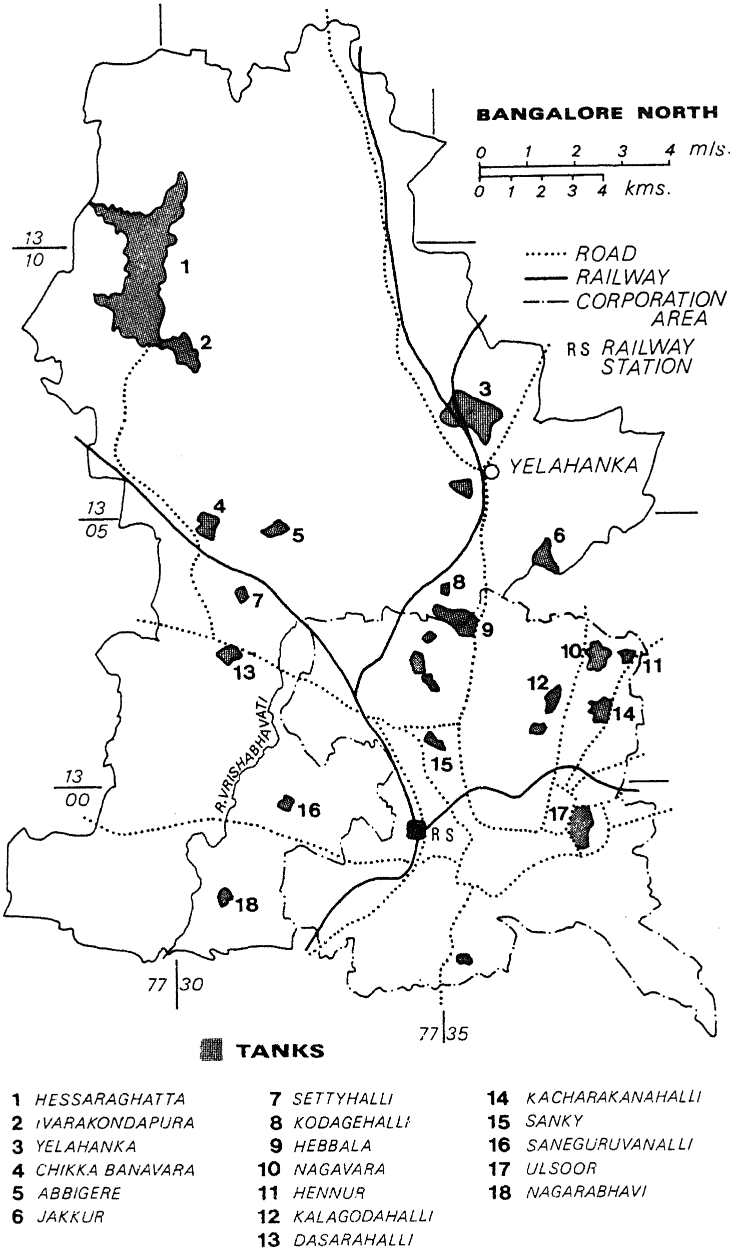

Figure 2. Freshwater habitats located in the North taluk of Bangalore.

During the survey, 4 species of prawns, one belonging to the family Palaemonidae (*Macrobrachium lanchesteri* (de Man); size range: 10–56 mm total length) and 3 species belonging to the family Atyidae (i. *Caridina rajadhari* (Bouvier); size range: 10–32 mm TL; ii. *C. nilotica* Roux var *bengalensis*; size range: 10–34 mm TL and iii. *C. weberi* var *sumatrensis* (de Man); size range: 10–30 mm TL) were collected.

Figure 2 represents the location of the freshwater habitats in the Bangalore North

Table 1. Distribution and abundance of prawns in the freshwater habitats of Bangalore North taluk. The numbers indicated in column 2 correspond to the habitats denoted in figure 2. In columns 3 and 4, only the ranges are represented.

Nature of the habitat	Habitat number	Density of prawns (Number/20 m^2)	Distribution (%)			
			M.l.	C.n.	C.r.	C.w.
A. *Perennial habitats*						
i. Rainfed; fairly deep in the centre; grassy vegetation at the shallow margins; open waters devoid of weeds; bottom sediment mostly made up of clay and sand	1, 9 and 15	45–333	47–82	18–51	0–18	<1–3
ii. As above but infested with *Eichhornia* sp.	3	273	<1	78	19	2
iii. As in i. but with rocky bottom	18	5	100	—	—	—
iv. As in i. or ii. but with considerable drainage of domestic/industrial effluents	17	—	—	—	—	—
B. *Seasonal habitats*						
i. Rainfed; grassy vegetation at the margins open waters devoid of weeds; bottom sediment made up of clay & sand	2, 5, 7, 8	15–483	8–100	0–44	0–27	0–35
ii. Rainfed; fairly deep with slushy bottom; with *Eichhornia* sp.	10	531	4	58	37	1
iii. Rainfed; shallow with slushy/rocky bottom; no aquatic vegetation in the margins	4, 6 and 11	—	—	—	—	—
iv. Shallow with considerable drainage of domestic sewage/industrial effluents	12, 13, 14 and 16	—	—	—	—	—

M.l.: *M. lanchesteri* (de Man); C.n.: *C. nilotica* (Roux) var *bengalensis*; C.r.: *C. rajadhari* (Bouvier); C.w.: *C. weberi* var *sumatrensis* (de Man).

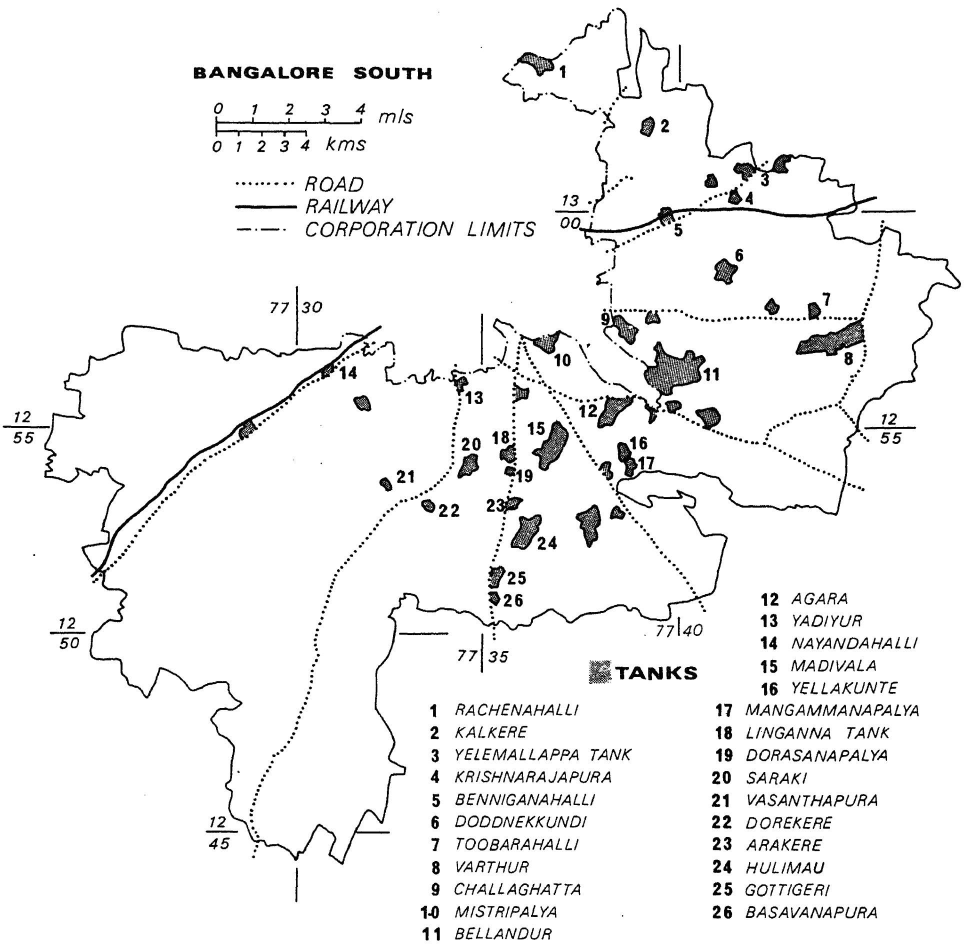

Figure 3. Freshwater habitats located in the South taluk of Bangalore.

taluk and table 1 gives the description of the distribution and abundance of prawns in relation to the nature of these habitats. It is evident that prawns are absent in habitats receiving domestic/industrial effluents and habitats with shallow margins and grassy vegetation appeared to support a rich fauna of these natantians. That the sewage has adverse effects on the survival/physiological energetics of food conversion in *M. lanchesteri* is reported earlier (Ponnuchamy *et al* 1980). It is quite likely that the microdistribution and abundance of both *Macrobrachium* sp. and *Caridina* spp. may be dependent on the nature of bottom sediment and/or aquatic vegetation (see Carpenter 1978).

The location of the freshwater habitats of Bangalore South taluk are represented in figure 3 and the details of the distribution and abundance of the prawn species in relation to the nature of these habitats is represented in table 2. As in the Bangalore North taluk, distribution and abundance of the prawns appear to be dependent on the absence of effluents, the type of bottom sediment and the presence of aquatic vegetation.

Table 2. Distribution and abundance of prawns in the freshwater habitats of Bangalore South taluk. The numbers indicated in column 2 correspond to the habitats denoted in figure 3. In columns 3 and 4, the ranges are represented.

Nature of the habitat	Habitat number	Density of prawns (Number/20 m^2)	Distribution (%)			
			M.l.	C.n.	C.r.	C.w.
A. *Perennial habitats*						
i. Rainfed; grassy vegetation in the margins; open waters devoid of weeds; bottom sediment mostly made up of clay and sand	4, 5, 15, 16, 21, 24 and 25	21–409	<1–71	17–42	0–67	0–58
ii. As above but infested with *Eichhornia* sp.	12	147	11	47	43	—
iii. As in i. but with rocky bottom	18	—	—	—	—	—
iv. As in i. and ii. but with considerable drainage of domestic sewage/industrial effluents	8 and 11	—	—	—	—	—
B. *Seasonal habitats*						
i. Rainfed; grassy vegetation in the margins; open waters devoid of weeds; bottom sediment mostly made up of clay and sand	1, 2, 6, 10, 14, 17 19, 22 and 23	3–443	3–100	0–90	0–61	0–40
ii. Rainfed; fairly deep with slushy bottom; infested with *Eichhornia*/*Hydrilla* sp.	3 and 20	8–12	0–32	59–69	0–29	2–9
iii. Shallow with slushy/rocky bottom; no aquatic vegetation in the margins	7 and 26	—	—	—	—	—
iv. Shallow with considerable drainage of domestic sewage/industrial effluents	9 and 13	—	—	—	—	—

Table 3 presents the data on the distribution and abundance of prawns in the freshwater habitats around Bangalore North and South taluks. It is again evident that the prawns prefer habitats devoid of effluents but those rich in clay or sandy sediment with grassy vegetation harbour a rich fauna of these prawns. From this it is apparent that the distribution of the freshwater prawns of Bangalore is primarily dependent on the nature of the habitat. While there appears to be not much variation in the distribution patterns of the different species in relation to the perennial or seasonal nature of the habitats, it is largely dependent on the type of bottom sediment, aquatic vegetation as well as the presence or absence of domestic/industrial effluents. That most of the habitats supported a fairly rich fauna of these 4 coexisting species is interesting.

4. Discussion

The genus *Palaemon* (= *Macrobrachium*) belonging to the family palaemonidae is known to be extensively distributed in tropical and sub-tropical regions of the world. While Ortman (1902) opined that the distribution of this genus has no value in the study of ancient geography, Tiwari (1955) has fairly satisfactorily explained certain hypothesis based on the then existing distribution of several members belonging to the genus. According to him, the genus *Palaemon* originated rather late in geographical history, probably in littotal (marine) areas and began migrating to freshwater. In fact, the family palaemonidae as a whole, has been considered to be in the process of migrating from marine to freshwater systems (Emery and Stevenson 1957; John Mary 1957). Based on the above consideration, Tiwari (1955) distinguished the coastal species of the genus from those inhabiting inland freshwater systems of India. However, a large number of species included by him in the latter group are known to be the migratory varieties, still dependent on saline medium, at least for purposes of breeding and completing their larval histories (see Panikkar 1967; Jhingran 1982). While few species have also been indicated to be distributed in ponds/tanks, Tiwari (1955) has mentioned nothing about the distribution of such species in the lentic habitats of the country. Such species complete their entire life-history in totally inland freshwater systems (see also Raman 1976). *M. lanchesteri* (de Man) which is widely distributed in the freshwater habitats in and around Bangalore is one such species which is fully established in closed freshwater systems and thrives in land-locked freshwater habitats (see Johnson 1967; Raman 1976 and present observations). Surprisingly, Tiwari (1955) has not included this species in his discussion on the distribution of the group, despite his own first record of the species from Nagpur and Baroda Aravallis and Gujarat Satpuras as early as 1947. The wide occurrence of this species in the land-locked freshwater systems of Bangalore, suggests that this species is one of the earliest to have migrated, perhaps from coastal waters and is now fully established in totally inland freshwater habitats away from the coast. The ability of *M. lanchesteri* to tolerate saline media to a certain extent, despite its long phylogenetic history in land-locked freshwater systems (see Ponnuchamy 1981), offers further evidence to its probable marine/estuarine ancestry.

M. lanchesteri (de Man) is a species of Malayasian origin (Lanchester 1901; Johnson 1967). The record of this Malayan species, from the Satpura trends of mountains (Nagpur and Baroda; Tiwari 1947) and Bangalore (present study) is of special interest. Based on the distribution of hill stream fishes of India, Hora (1944) put forth the hypothesis that, waves of migrations of Malayan fauna passed over the Eastern

Table 3. Distribution and abundance of prawns in the freshwater habitats around Bangalore North and South taluks.

Nature of the habitat	Name of the habitat	Density of prawns (Number/20 m^2)	Distribution (%)			
			M.l.	C.n.	C.r.	C.w.
A. *Perennial habitats*						
i. Rainfed; fairly deep with grassy vegetation in the margins/some aquatic weeds.	Mantapa and Kariyappanapalya	249–286	26–51	41–58	9–16	0–<1
ii. As above but without aquatic vegetation	Hoskere	—	—	—	—	—
B. *Seasonal habitats*						
i. With grassy vegetation in the margins, open waters devoid of weeds	Dodda Gubbi, Jigani and Koppa	10–148	21–90	10–40	0–46	0–<1
ii. As above but infested with aquatic weeds	Kannur	800	53	22	25	<1
iii. As in i. but with no marginal vegetation	Hoskote	10	29	71	—	—
iv. As in i. and ii. but with considerable drainage of domestic sewage/industrial effluents	Vengaiah	—	—	—	—	—

Himalaya and then to the Western Ghats and the extreme South of India. From time to time, more evidences have been sighted in support of this hypothesis (Menon 1951; 1977; Silas 1952). The discontinuous distribution of *M. lanchesteri* (de Man) extending from the Satpuras to the South of India (Bangalore) lends further support to this hypothesis.

In regard to systematics, the genus *Caridina* is known to be one of the notoriously difficult genera of Atyidae (Holthuis 1965), which is a major family of decapods largely comprising freshwater prawns of warmer tropics. The probable origin of these primitive caridean prawns is traced to Tethys, a shallow-water tropical sea during the cretaceous (Banarescu 1973) and they are known to be most common in Indo-pacific and Caribbean regions (Johnson 1963). The family is usually described as restricted to freshwaters, although a number of species are known to inhabit 'anchialine'* pools with a salinity as high as 30% (Holthuis 1973).

Several lines of evidence suggest that unlike the Palaemonidae the Atyidae are ancient inhabitants of freshwater (Roxo 1940; Beurlen 1950). Since salt tolerance or life in proximity to the sea is no criterion of immediate marine ancestry (Ortman 1894; Schminke 1975), especially in a large family like Atyidae, with no present day marine representatives (Fryer 1977), it is probable that the members of the family have a long history in freshwater systems.

From time to time, several species of the genus *Caridina* have been reported from various Indian freshwater habitats (Natarajan 1942; Chinnayya 1968; Tiwari and Pillai 1968; Dutt and Ravindranath 1975; Ravindranath 1977). Of the species so far reported from Indian freshwaters, some are riverine varieties, while others have been recorded from smaller closed water bodies (Ponds and ditches). The 3 species of *Caridina* distributed in and around Bangalore are the confirmed land-locked varieties and appear to be discontinuously distributed in the inland freshwater systems of India. However, since authentic taxonomic identifications of the hitherto recorded varieties of the genus are lacking and the distribution of the different species of the genus, in Indian freshwater habitats is not fully known, a discussion on the distribution pattern of these species has not been attempted presently. The species of *Caridina* recorded presently reproduce in closed freshwater systems and their development is also abbreviated (i.e. eggs hatch into zoea larvae; see Ponnuchamy *et al* 1979; Rao *et al* 1981), provides additional ecological evidences for the hypothesis that the species are ancient inhabitants of freshwater. The occurrence of one or all the species of Atyids in most of the local habitats and their coexistence with a palaemonid (*M. lanchesteri*), suggests that, the genus *Caridina* and the 3 species included under it are advanced and diversified atyids, capable of competing with palaemonids (Walker 1972; Carpenter 1977). The key to their ability to have done so perhaps lies in their extremely specialised and efficient means of food collection (Fryer 1977; Ponnuchamy *et al* 1984), which permits the handling and utilization of minute food particles. However, the irregular pattern of distribution of these atyids as observed presently, as compared to that of *Macrobrachium*, suggests that their microdistribution in the habitats may be regulated either by specific environmental factors, nature of bottom sediment or the distribution of aquatic plant species (see Kemp 1917; Carpenter 1978).

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*'Anchialine' denotes a freshwater habitat with no surface connection with the seas but which contains saline water fluctuating with the tides.

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Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours

MOHD IDRIS and ISHWAR PRAKASH

Coordinating and Monitoring Centre for Rodent Research and Training, Central Arid Zone Research Institute, Jodhpur 342 003, India

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Abstract. Results of experiments conducted in T-maze to probe the functional role of conspecific odours indicated that whereas the male Indian gerbil, *Tatera indica indica* invariably prefers odours (from their body, that of the sebum exudation of scent marking gland, urine and faecal matter) of strange males, the females with and devoid of the scent marking gland show slight preference for male odours. However, all the categories of *Tatera indica* placed their preference for their own odours at the lowest. This behaviour suggests that the role of scent marking to label the habitat for its own use or to signal 'home' to the marking animal may not be the sole function of scent marking in this species. It is quite possible that in the desert grassland, scent marking is being used for maintaining a minimum distance between two animals or pairs, as exhibited by the diversity in the occurrence of the scent gland in the field (pair-tolerant) and urban (gregarious social organisation) populations of *Tatera indica*. This type of spatial distribution, maintained by scent marking behaviour, may be important to withstand the low carrying capacity of the aridland which suffers from almost a perpetual paucity of food.

Keywords. Cue; olfactory communication; scent marking; social behaviour; T-maze; urine marking.

1. Introduction

The Indian gerbil, *Tatera indica indica* (Hardwicke) is one of the predominant rodents found in the Thar desert. It occupies two distinct habitats i.e. desert grasslands and urban settlements. In the former situation it lives singly or in pairs in burrows situated quite far away from each other. In the urban habitat of Bikaner town (28° 0′N, 73° 17′E) where they are mostly found in the streets, their social organisation is gregarious and 4–70 gerbils occupy a single burrow system. In the field population, the ventral scent marking gland occurs in 91·4 % males and 38·5 % females whereas in the urban one, in 85·6 % males and 3·2 % females (Idris and Prakash 1983). However, the rate of scent marking in the gerbils of latter community is relatively of low order ($P < 0{\cdot}05$, unpublished results). We are seeking the reason for these differences in the frequency of occurrence of the scent marking gland and behavioural implication in the two types of populations of *T. indica* with a view to probe the functional role of conspecific odours in their social organisation.

2. Methods

Wild *T. indica* were collected from the scrub grasslands around Jodhpur (26° 18′N, 73° 01′E). The sexually mature rodents were divided into 3 groups of 12 animals each:

male possessing the scent marking gland, female possessing the gland and female devoid of the gland. Each animal of a group was released in the control chamber of a T-maze of which 3 arms carried different odours provided in 3 combinations. The released rodent was free to move and explore any of the arms. The number and duration of visit to each arm, frequency of sniffing, grooming, scent marking, urine marking, urination and defecation were recorded for 30 min at their maximum activity period (20·00–22·00 hr) under dim red light. Such observations on each rodent were made for 12 days.

The arms attached to the T-maze were prepared for exposure to new animals by separately lodging the 3 types of *T. indica* (male and female with gland, and female without gland) for 24 hr. During this duration the animals possibly saturated the maze arm with the odours from their body, scent marking gland, urine and faecal matter.

3. Results

The activity pattern pertaining to grooming, urination and defecation exhibited in various odour environments by the three classes of *T. indica* (male, female with gland, and female without gland) was not significantly different, therefore they have not been taken into account of results summarised in tables 1–3.

3.1 *Behavioural responses of male T. indica*

The male *T. indica* preferred the odour environment of strange male as indicated by the frequency of their sniffing ($P < 0{\cdot}01$; $P < 0{\cdot}001$), scent marking ($P < 0{\cdot}01$), urine marking ($P < 0{\cdot}001$) and frequency of visits to the arm ($P < 0{\cdot}05$) as compared to their activity in clean arm of the T-maze (table 1). However, a comparison of activities in environ of strange male, strange female and clean box revealed that the difference in frequency in former two environments was not significantly different. It further indicates that choice of male *T. indica* for strange male and female odours is more or less equal.

Another point which is clearly brought out by the behavioural responses of male *T. indica* is that it did not prefer its own odour even in comparison to that of clean environment (table 1) but the duration of time during which the males stayed in own-odour environment is significantly more ($P < 0{\cdot}05$) as compared to the arms carrying other odours.

3.2 *Behavioural responses of female T. indica possessing gland*

The females possessing scent marking gland also preferred the clean environment over own odour as their sniffing and urine marking activities were significantly less ($P < 0{\cdot}05$) in the maze arm carrying own odour (table 2). These females, however, differ from male *T. indica* in spending significantly less ($P < 0{\cdot}05$) time in the arm carrying their own odour as compared to the clean arm. Female possessing scent marking gland were found to be more attracted towards the odour of strange female possessing the gland (table 2), as indicated by frequency of scent marking and number of visits

Table 1. Behavioural responses (Mean ± SE) of male *T. indica* to different conspecific odours.

	Behavioural acts in 30 min period					
Conspecific odour choice	(N)	Sniffing	Ventral marking	Urine marking	No. of visits	Duration of visits (seconds)
Own odour		5·83±1·07 NS	2·33±0·43^a	1·50±0·31 NS	9·91±0·96 NS	835·00±47·40^a
Strange male	12	10·91±1·23^a	6·00±0·71^b	2·08±1·72 NS	13·68±1·37 NS	477·00±54·94 NS
Clean box		6·66±1·21	3·41±0·41	1·58±0·35	11·58±2·50	488·25±24·21
Strange male		9·08±1·27^c	4·91±0·27^a	2·33±0·35^a	10·16±0·73^a	588·75±61·68
Strange female with gland	12	5·16±0·92 NS	4·35±0·68^a	2·33±0·35^a	9·91±5·70 NS	659·00±42·59 NS
Clean box		4·25±0.64	2·63±1·28	1·00±0·36	8·38±0·70	560·41±64·57
Strange male		7·75±0·83 NS	5·66±0·59^c	2·27±0·27 NS	12·33±1·10^a	708·55±55·83^a
Strange female with gland	12	7·45±0·73	3·00±0·37 NS	1·58±0·35 NS	9·33±6·49^a	614·33±38·78^a
Strange female without gland		5·83±0·63	2·08±0·35	2·21±0·29	7·90±0·57	477·00±47·95

Student's t test, level of significance. $^aP < 0·05$; $^bP < 0·01$; $^cP < 0·001$; NS = Not significant.

Table 2. Behavioural responses (Mean ±SE) of female *T. indica* with gland to different conspecific odours.

Conspecific odour choice	(N)	Behavioural acts in 30 min period Sniffing	Ventral marking	Urine marking	No. of visits	Duration of visits (seconds)
Own odour		8·66 ± 1·05^a	1·86 ± 0·44 NS	1·26 ± 0·32^a	11·16 ± 0·80 NS	422·66 ± 45·08^b
Strange female with gland	12	14·55 ± 1·36^b	3·00 ± 0·53 NS	2·41 ± 0·41 NS	16·50 ± 1·01^a	713·50 ± 57·30 NS
Clean		11·83 ± 0·96	2·30 ± 0·47	2·72 ± 0·48	13·58 ± 1·13	663·83 ± 67·47
Own odour		10·56 ± 0·81	1·66 ± 0·48	1·75 ± 0·42	8·83 ± 0·70	453·83 ± 53·53
Strange female with gland	12	12·00 ± 1·31 NS	3·58 ± 0·58^a	1·41 ± 0·37 NS	12·75 ± 1·21^a	692·00 ± 70·73^a
Strange female without gland		13·83 ± 0·92^a	1·66 ± 0·53 NS	1·16 ± 0·53 NS	9·50 ± 1·19 NS	654·16 ± 37·27^a
Strange female with gland	12	9·83 ± 1·83 NS	2·00 ± 0·38 NS	1·58 ± 0·35 NS	9·15 ± 0·77 NS	649·88 ± 66·61^a
Strange female without gland		7·25 ± 2·43	1·58 ± 0·33	0·83 ± 0·33	7·25 ± 0·86	482·33 ± 57·88
Strange male		10·83 ± 2·45 NS	2·58 ± 0·57 NS	0·76 ± 0·24 NS	12·08 ± 1·32^b	667·83 ± 49·37^a

Student's *t* test, level of significance. $^aP < 0·05$; $^bP < 0·01$; NS = Not significant.

Table 3. Behavioural responses (Mean ± SE) of female *T. indica* to different conspecific odours.

Conspecific odour choice	(N)	Behavioural acts in 30 min. period				
		Sniffing	Ventral marking	Urine marking	No. of visits	Duration of visits (seconds)
Own odour		5·88 ± 1·39 NS	—	2·08 ± 1·50 NS	8·33 ± 0·92 NS	400·25 ± 68·67^c
Strange female without gland	12	12·50 ± 1·46^a	—	3·00 ± 0·57 NS	11·33 ± 1·21 NS	692·50 ± 47·23 NS
Clean		8·58 ± 1·70	—	2·69 ± 0·47	8·50 ± 0·87	706·38 ± 56·85
Own odour		10·66 ± 1·09	—	1·33 ± 0·35	8·58 ± 1·07	589·00 ± 45·48
Strange female with gland	12	13·83 ± 1·32 NS	—	3·58 ± 0·52^c	11·09 ± 0·79^a	450·40 ± 34·82^a
Strange male		15·41 ± 5·63^a	—	4·66 ± 0·84^b	12·83 ± 1·20^a	761·58 ± 47·43^a
Strange female without gland		12·60 ± 1·39 NS	—	1·50 ± 0·31 NS	10·00 ± 1·12 NS	533·00 ± 44·54 NS
Strange female with gland	12	12·45 ± 1·23	—	1·41 ± 0·60	13·00 ± 0·44	462·41 ± 22·28
Strange male		13·83 ± 0·13 NS	—	4·75 ± 0·59^c	13·25 ± 1·35 NS	805·41 ± 38·61^c

Student's *t* test, level of significance. $^a P < 0{\cdot}05$; $^b P < 0{\cdot}01$, $^c P < 0{\cdot}001$; NS = Not significant.

($P < 0.05$) as compared to the responses to own odours. But no sooner the odour of strange male was available (table 2), the preference was for the strange male though it never enhanced to a significant level than that in the arm carrying odours of female possessing the gland. In this hierarchy of preference, the body odours of the female devoid of the scent gland were rated at the lowest level.

3.3 *Behavioural responses of female T. indica without gland*

The behaviour of females without the gland was almost similar to that of females possessing the gland, own odour was rated lowest (table 3), male odours and that of female with gland were equally preferred with an edge for the female odours (table 3).

4. Discussion

The results indicate that the male *T. indica* invariably prefers the odour of strange individual of their own sex but its scent marking response in presence of odours of female possessing the gland was also equivalent to that of strange male. The female possessing the gland, rate the strange male odour and strange female odour almost at an equal level. The female devoid of the gland also preferred the odours of strange males but when the choice was between the odours of female with the gland and male, the difference between their rating was not significant. The individuals belonging to 3 categories of *T. indica* exhibited lowest preference for their own odour.

Both types of female were attracted to male odours as indicated by the frequency of sniffing, scent marking, urine marking and the number and duration of visits to the arm containing the male odour. However, the males were attracted towards female odours only when those of a strange male were not available to them.

The odours which an animal (*T. indica*) can possibly impregnate in an environment (the maze arm in this study) are (i) body odour, (ii) odour of the sebum of the ventral scent marking gland, (iii) odour of urine and (iv) odour of the faecal matter. It is not possible to say which one of these is involved in the attraction. However, if the responses of female *T. indica* devoid of scent gland (table 3) are considered, it would appear that the sebum odour plays a major role in the olfactory communication. Male *Meriones tristrami* also show attraction towards odours from strange males whereas females do not show a consistent preference (Thiessen *et al* 1973). Likewise female, *M. unguiculatus* does not show any significant preference for the odour of males in single choice tests; but when tested together, the female show a slight preference for own sex odour (Thiessen *et al* 1970).

It is known that female *T. indica* possessing scent gland (Prakash and Idris 1982), enhance scent marking and urine marking frequency in the presence of urine and sebum odours of strange female in oestrus (Prakash and Idris 1982). The males also do so in similar environment (Idris and Prakash 1983), indicating the involvement of odours in the reproduction activity. Our results suggest that males prefer female odours in the absence of their own sex odour (table 1). Females also show a similar preference (table 2). The observations confirm the involvement of odours in the olfactory communication during the reproductive activity in *T. indica* (Kumari and Prakash 1983). The odours have also been found to be broadly correlated with dominance hierarchy in *T. indica* (Idris and Prakash 1985). However, the situation is not clear with respect to the females of *T. indica*, possessing the scent marking glan and those devoid of it since in the latter, urine marking, which is subsidiary to sebu marking in other categories of *T. indica*, plays the prime role in olfactory communication (Idris and Prakash 1983).

The low rating of own-sex odours by the 3 categories of *T. indica* (tables 1–3) canno be properly explained. Earlier, we have interpreted that the function of scent markin in this species is more of a familiarisation nature to label the habitat for its own use an in orientation or to signal "home" to the marking animal (Idris and Prakash 1982 Other workers (Ewer 1973; Mykytowycz 1970; Ralls 1971) have also suggested thi function, who believe that the scent marks provide a sense of security and increas confidence of the animals in the general area of their home range. Such a confidence enhancing effect is, however, not supported by the results of our study; in that *T. indic* does not prefer its own scent in comparison to strange animals of own sex or opposit sex. In fact various categories of *T. indica* rated their own odour even below clean ar of the T-maze. The behaviour of *T. indica* in this respect is quite perplexing unless it i assumed that the frequency of various behavioural activities (tables 1–3) of categories of *T. indica* in their own odour arm of T-maze provided to them 'familia odours environment and hence their scent marking activity in these arms was low a compared to others (carrying sebum odour of strange male or female) in which the enhanced their scent and urine marking activities to saturate them with their ow odours to make them 'familiar'.

It is quite possible, however, that scent marking cues may be used by *T. indic* inhabiting the scattered population in open grasslands for maintaining a minimu distance between two animals or pairs. This concept gets support from our finding (Idris and Prakash 1982) about the diversity in the frequency of occurrence of the scen gland in two types of populations living in different social organisation. Th maintenance of a minimum distance between two pairs may be necessary not only fro the social interactions point of view but also in consonance with the carrying capacit of the arid land which suffers from almost a perpetual paucity of food.

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A study on the effects of certain abiotic factors on the activity of *Adoretus epipleuralis* Arrow (Coleoptera: Scarabaeidae)

ROHTASH CHAND

Department of Biological Sciences, Birla Institute of Technology and Science, Pilani 333 031, India

Present address: Department of Zoology, Kurukshetra University, Kurukshetra 132 119, India

MS received 25 January 1985

Abstract. The effect of certain abiotic factors viz. temperature relative humidity and soil moisture has been revealed on the activity of *Adoretus epipleuralis* Arrow. This beetle was caught with the help of Pilani type light trap operated daily for 2 years (1976, 1977) from dusk to dawn at Pilani (Pilani is on the North-eastern side of Jhunjhunu District of Shekhawati Region in semi-arid zone of Rajasthan, India. Its geographical position is 28°20′ N latitude and 75°35′ E longitude and 330 mase.). To ascertain in mathematical terms the extent of relationship between the prevailing weather conditions and the light trap catches of *Adoretus epipleuralis*, regression analysis, partial regression analysis, multiple linear regression analysis and β-coefficient analysis have been exercised.

Keywords. Abiotic factor; catch; flight activity.

1. Introduction

The study shows that *Adoretus epipleuralis* responds well to the soil moisture component of the abiotic environment (correlation coefficient $r = 0{\cdot}7991$, regression coefficient $= 0{\cdot}2215$ and β-regression coefficient is $= 0{\cdot}5716$). This response is the highest in comparison to its response to other weather factors and is closely followed by minimum temperature (correlation coefficient $= 0{\cdot}6439$, regression coefficient $= 0{\cdot}0706$, and β-regression coefficient $= 0{\cdot}4947$). The rest of the factors are having either negative or just negligible influence.

Nothing is known about its ecology in the Indian context. Practically no work has been done on *A. epipleuralis* in Rajasthan. The present work will help unfold certain ecological facts about *A. epipleuralis*, a voracious forest pest.

2. Material and methods

The dependent factor i.e. the abundance of *A. epipleuralis* was recorded daily for 2 years (1976, 1977). This has been accomplished by operating the Pilani type light trap (Kundu *et al* 1961; Jakhar 1969; Narula 1969; Bandyopadhyay 1970; Naik and Kundu 1977; Chand 1979) daily from dusk to dawn.

The independent abiotic factors i.e. 8.30 AM relative humidity, 5.30 PM relative humidity, mean relative humidity, minimum temperature, maximum temperature, mean temperature and rainfall were also recorded from the meteorological sub-station of Government of India which is being maintained over here at Pilani. Readings of the Stevensen's Screen were taken twice a day, once at 8.30 AM and again at 5.30 PM.

As far as soil moisture factor is concerned, 3 samples of soils were taken from the vicinity at 3 depths (0–5, 10–15 and 25–30 cms) just after switching on the light trap and completing the other necessities to make it functional. All the samples were brought to the laboratory and processed in IR Moisture Balance (Toshniwal) to calculate the moisture contents on dry matter weight basis. The following given formula was used:

$$\text{Moisture } (\%) = \frac{100 \times P}{100 - P}$$

where P is the reading read directly on the balance. The samples were put to a temperature of 105°C for a duration of half an hour. The mean of the 3 samples were recorded for further calculations.

2.1 *Interpretation of the catch*

Williams (1940) opined that "the catch in the light trap in any night is a sample, as unbiased as possible, of the night flying positively phototrophic insects which are active in the neighbourhood of the trap". According to Williams (1940):

Catch α-activity × population.

Since the flight activity of various insects is again dependent on the effect of various weather components, the total activity is defined as:

Total activity = activity due to temperature = activity due to humidity etc.

Catch α (activity due to temperature × activity due to humidity etc.) × population.

Now, as a result of the values being expressed in log catch by proportional changes

Log catch = (log activity temperature + log activity humidity etc. + log population).

The log catch is, therefore, made up of portions due to the effect on the activity of different climatic factors plus a portion decided by the total population available for sampling. It may be pointed out here that light trap captures or catches have been used to specify the individuals caught, of a particular species, in the light trap.

The numbers of all the captures of *A. epipleuralis* have been converted to log values from which all mathematical calculations have been done (Williams 1937). Here 5-day means of the log values of the captures have been used for the purpose of calculations (Jakhar 1969; Narula 1969; Bandyopadhyay 1970; Naik and Kundu 1977; Chand 1979). Since the value of log of zero is minus infinity, one has been added to all catches before taking their log values (Williams 1937).

In order to estimate the level of correlationship between activity of *A. epipleuralis* (captures in the light trap) and the selected abiotic factors simple regression analysis and partial regression analysis has been done following Williams (1937, 1939, 1941), Jakhar (1969), Narula (1969), Bandyopadhyay (1970) and Chand (1979).

Since a number of factors affect the flight activity of insects, the contribution due to each of these can be assessed with the help of multiple regression models. Multiple regression analysis has been done following Laebo (1968). It may be mentioned here that the two abiotic factors viz. mean temperature and mean relative humidity have been excluded from the models as they are functions of maximum and minimum temperature, and 08.30 hr and 17.30 hr relative humidity parameters respectively. Once the temperature and relative humidity factors are defined in the models, their means are automatically defined.

Since the β-regression coefficients provide a more accurate information of the relative importance of the independent variables, such an analysis has also been taken following Laebo (1968).

3. Results

The 8 regression equations obtained on *A. epipleuralis* due to the effect of various 8 abiotic factors for the period January to December both years (1976, 1977) taken together are given below:

(i)	Soil moisture	$\hat{Y} = 0{\cdot}2215X + 0{\cdot}1731$:($r = 0{\cdot}7991$, $P = 0{\cdot}001$)
(ii)	8.30 AM relative humidity	$\hat{Y} = 0{\cdot}0232X - 1{\cdot}0910$:($r = 0{\cdot}4293$, $P = 0{\cdot}001$)
(iii)	5.30 PM relative humidity	$\hat{Y} = 0{\cdot}0229X - 0{\cdot}4015$:($r = 0{\cdot}4320$, $P = 0{\cdot}001$)
(iv)	Mean relative humidity	$\hat{Y} = 0{\cdot}0248X - 0{\cdot}8467$:($r = 0{\cdot}5021$, $P = 0{\cdot}001$)
(v)	Maximum temperature	$\hat{Y} = 0{\cdot}0439X - 0{\cdot}8384$:($r = 0{\cdot}2989$, $P = 0{\cdot}01$)
(vi)	Minimum temperature	$\hat{Y} = 0{\cdot}0706X - 0{\cdot}5956$:($r = 0{\cdot}6439$, $P = 0{\cdot}001$)
(vii)	Mean temperature	$\hat{Y} = 0{\cdot}0677X - 1{\cdot}0780$:($r = 0{\cdot}5173$, $P = 0{\cdot}001$)
(viii)	Rainfall	$\hat{Y} = 0{\cdot}1398X - 0{\cdot}3428$:($r = 0{\cdot}6719$, $P = 0{\cdot}001$)

The extent of increase or decrease in various climatic factors required to double catch is as below:

Soil moisture (%)	= 1·36 %
8.30 AM relative humidity	= 12·97 %
5.30 PM relative humidity	= 13·14 %
Mean relative humidity	= 12·13 %
Maximum temperature	= 14·26°C
Minimum temperature	= 6·85°C
Mean temperature	= 4·44°C
Rainfall	= 2·15 mm

The values of partial correlation coefficients of log catch on various climatic factors are given in table 1.

Multiple regression equation derived is given below:

$$\hat{Y} = 0{\cdot}4262 + 0{\cdot}1584X_1 + 0{\cdot}0052X_2 - 0{\cdot}0018X_3 - 0{\cdot}0150X_4 + 0{\cdot}0542X_5 - 0{\cdot}0019X_6\text{: } (r = 0{\cdot}6705,\ P = 0{\cdot}001).$$

Table 1. Partial correlation coefficients of log catch for *A. epipleuralis* on various climatic factors for the period January 1976–December 1977

Independent variable \ Eliminated variable	Soil moisture	8.30 AM relative humidity (%)	5.30 PM relative humidity (%)	Mean relative humidity (%)	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Rainfall (mm)
Soil moisture	—	0·2251	0·2167	0·2288	0·2195	0·1777	0·1977	0·2354
8.30 AM relative humidity	−0·0012	—	−0·0085	−0·0373	0·0345	0·0221	0·0281	0·0078
5.30 PM relative humidity	0·0011	0·0288	—	0·0373	0·0271	0·0179	0·0224	0·0088
Mean relative humidity	0·0001	0·0576	−0·0170	—	0·0323	0·0210	0·0203	0·0092
Maximum temperature	0·0409	0·0798	0·0644	0·0729	—	−0·1192	−0·2646	0·0448
Minimum temperature	0·0406	0·0691	0·0611	0·0544	0·1452	—	0·2544	0·0515
Mean temperature	0·0436	0·0780	0·0660	0·0715	0·2906	−0·2384	—	0·0523
Rainfall	−0·0121	0·1256	0·1144	0·1183	0·1404	0·1066	0·1233	—

The β-regression coefficient values due to the various climatic factors are:

Soil moisture (%) = 0·5716
8.30 AM relative humidity (%) = 0·0969
5.30 PM relative humidity (%) = −0·0418
Maximum temperature (°C) = −0·1025
Minimum temperature (°C) = 0·4947
Rainfall (mm) = −0·0093

Based upon the multiple regression analysis technique, the level of increase required in a particular parameter in order to double the catch of *A. epipleuralis*, keeping all other parameters constant at that time are given below:

Soil moisture (%) = 1·89
8.30 AM relative humidity = 57·80
5.30 PM relative humidity = −167·20
Maximum temperature (°C) = − 20·06
Minimum temperature (°C) = 5·55
Rainfall (mm) = −158·40

4. Discussion

It is evident, based upon the values of simple correlation coefficients, that the highest value is obtained due to soil moisture ($r = 0{\cdot}7991$) followed by rainfall ($r = 0{\cdot}6719$); minimum temperature ($r = 0{\cdot}6439$); 5.30 PM relative humidity (%) ($r = 0{\cdot}5320$); mean temperature ($r = 0{\cdot}5173$); mean relative humidity (%) ($r = 0{\cdot}5021$); and 8.30 AM relative humidity (%) ($r = 0{\cdot}4293$) all significant at $P = 0{\cdot}001$ level. Further the most influential values of regression coefficients are obtained on soil moisture (0·2215); rainfall (0·1398) and minimum temperature (0·0706). It is also evident that the minimum level of increase required in a certain parameter in order to evoke a response in the log catch of *A. epipleuralis* to double itself; is found in case of soil moisture (1·36 %) followed by rainfall (2·15 mm) and mean temperature (4·44°C). It is also clear that this influential mean temperature effect on the log catch of *A. epipleuralis* is being exercised indirectly through minimum temperature (level of increased temperature required is 6·85°C) whereas in case of maximum temperature this value is 14·26°C. So according to the results of simple regression analysis it seems that soil moisture, rainfall and minimum temperature are more influential in that order. It is also clear that the relative humidity parameters are exercising almost equal influential role. However, maximum temperature seems to play an insignificant role in determining the log catch of *A. epipleuralis* ($r = 0{\cdot}2989$, $P = 0{\cdot}01$).

The salient feature of partial regression analysis is that soil moisture acts most positively on the log catch of *A. epipleuralis* (table 1).

The coefficient of multiple correlation is 0·8705 which is significant at $P = 0{\cdot}001$ level thus 75·78 % variability in the log catch is associated for by a linear combination of soil moisture (X_1), 8.30 AM relative humidity (X_3), minimum temperature (X_4), maximum temperature (X_5) and rainfall in mm (X_6) according to the following regression:

$$\hat{Y} = 0{\cdot}4262 + 0{\cdot}1584X_1 + 0{\cdot}0052X_2 + 0{\cdot}0018X_3 - 0{\cdot}9150X_4 + 0{\cdot}0542X_5 - 0{\cdot}0019X_6,$$

which may be interpreted to mean that estimated log catch increases or decreases by a value equal to the net regression coefficients of the respective climatic factor as shown in the equation.

From the absolute values of β-coefficients it is apparent that the order of relative importance of the different climatic factors is as follows:

> Soil moisture (0·5716), Minimum temperature (0·4947), Maximum temperature (−0·1025), 8.30 AM relative humidity (0·0969), 5.30 PM relative humidity (−0·0418), rainfall (−0·0093).

From the above it is evident that soil moisture and minimum temperature are the two most influential climatic factors in determining the log catch of *A. epileuralis* and maximum temperature tends to play a negative role whereas all others are insignificant.

Acknowledgements

The author is grateful to Dr C R Mitra and Dr M C Joshi for providing facilities. Thanks are also due to UGC New Delhi for providing financial assistance.

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Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton)*

K M RENUKARADHYA and T J VARGHESE
University of Agricultural Sciences, College of Fisheries, Mangalore 575 002, India

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Abstract. In order to determine the optimum protein requirement in the feeds for *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton), isocaloric pelleted feeds containing 20 %, 30 %, 40 % and 45 % protein were formulated using casein as the main source of protein. The best growth of catla and rohu was observed when fed on feeds containing 30 % and 40 % protein. The growth was poorer when fed on the diet containing 20 % protein and poorest when fed on the feed containing 45 % protein. The results revealed that the protein requirement of both catla and rohu was around 30 %. It also indicated that more than the optimum level of protein in feeds has an adverse effect on the growth of the species investigated.

Keywords. Protein requirement; *Catla catla*; *Labeo rohita*.

1. Introduction

Protein is the most important nutrient promoting growth in animals. As protein is the costliest among the various ingredients used for the preparation of fish feeds, it is necessary to ascertain the quantitative requirement of dietary protein in order to reduce the cost of feeds. A few reports are available on the protein requirement of fishes like Plaice (Cowey *et al* 1972), rainbow trout (Delong *et al* 1958; Zeitoun *et al* 1973), grass carp (Dabrowski 1977), Tilapia (Davis and Stickney 1978), milk fish (Lim *et al* 1978) and common carp (Varghese *et al* 1976). Delong *et al* (1958) were the first to use Casein-gelatin diets in studies on optimal protein level of fish feeds. Varghese *et al* (1976) and Dabrowski (1977) reported the protein requirement of common carp and grass carp to be 31 % and 41–43 % respectively. Information on the protein requirements of Indian major carps is very meagre. The only information available on this aspect is from the experiments of Sen *et al* (1978) and Srikrishnadhas *et al* (1980). In view of this, it was felt necessary to investigate the protein requirement of commonly cultivated carps of India. As a part of this programme, protein requirement of catla and rohu was studied in the present experiment.

2. Material and methods

2.1 *Feed*

Four experimental feeds containing 20 %, 30 %, 40 % and 45 % protein were used in this study. The major ingredients used for the formulation of the feeds were ricebran, groundnut oil cake, tapioca powder and casein, which served as the main source of protein. Cellulose and the standard mineral mixture prepared in the laboratory were also added to the experimental diets. Table 1 gives the proportion of various ingredients used for the formulation of different experimental diets, their protein content and calorie values. The ingredients purchased in powder form were sieved through ISI standard mesh No. 1 to get particles of uniform size, hand kneaded with sufficient quantity of water and made into a dough. The dough was then autoclaved in a closed aluminium container at 105°C for 30 min. Afterwards, the dough was cooled rapidly and extruded in the form of noodles, using a mechanical noodle making machine having a perforation diameter of 3 mm in the die. The noodles were sundried till the moisture content was reduced to less than 10% and were broken manually to a size of about 1 cm. The dried pellets were packed in heavy duty plastic bags and stored in a wooden shelf. The four feeds used were designated as follows for convenience.

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*Formed part of the M.F.Sc. thesis submitted by the senior author to the University of Agricultural Sciences, Bangalore.

(i) Pellet A (PA) (with approximately 20% protein content).
(ii) Pellet B (PB) (with approximately 30% protein content).
(iii) Pellet C (PC) (with approximately 40% protein content).
(iv) Pellet D (PD) (with approximately 45% protein content).

2.2 *Growth studies*

2.2.1 *Rearing facility and preparation of cisterns:* Twelve similar cisterns located in the fish farm of College of Fisheries were used for the experiment. Each cistern measuring 25 m^2 (5 × 5 m) had a soil bed of about 10 cm thickness. The cisterns were drained completely and dried prior to the beginning of the experiment. The cisterns were filled with water to a depth of 65 ± 5 cm. This level was maintained throughout the experimental period.

2.2.2 *Stocking and rearing:* Fingerlings of catla and rohu, raised from spawn obtained through hypophysation, were used for the experiment. All the 12 cisterns were uniformly stocked on 29th October 1982 with 8 fingerlings each of catla and rohu. Care was taken to ensure uniformity with regard to size of fingerlings. The total weight of each species stocked in different experimental cisterns was kept uniform. Fishes were fed once daily in the morning at the rate of 5% body weight.

Table 1. Proportion of various ingredients used in the formulation of different pelleted feeds.

Feed ingredients Type of Pellet	Casein (%)	Groundnut oil cake (%)	Rice bran (%)	Tapioca (%)	Minerals (%)	Cellulose (%)	Total	Energy value (Kcal)
Pellet A	6·30	24·00	40·00	28·70	1·00	—	100·00	316·05
Pellet B	17·00	24·00	40·00	18·00	1·00	—	100·00	316·08
Pellet C	28·10	24·00	40·00	6·70	1·00	0·20	100·00	316·08
Pellet D	33·50	24·00	40·00	0·78	1·00	0·72	100·00	316·07

2.2.3 *Sampling:* Growth data was obtained by sampling the stocked fishes every week. On the sampling days, water pH, atmospheric temperature and water temperature were recorded. Plankton and water samples were also collected from the cisterns and brought to the laboratory for analysis.

2.2.4 *Measurement and analysis—Fish growth:* A minimum of four specimens of each species were collected at random from each cistern and total weight of fishes recorded during every sampling, from which the average weight of each individual was calculated. On the day of termination (4 February 1983), all the surviving fishes were collected by dewatering the cisterns and their total weight recorded.

2.2.5 *Water analysis:* Water samples collected on sampling days were analysed for various physico-chemical parameters and the water quality was found to be suitable for fish life throughout the experimental period.

Since the plankton collected from the cisterns was negligible in quantity, the same was not considered for the study. The growth response of catla and rohu to feeds having different levels of protein was tested statistically by the analysis of variance technique (Snedecor and Cochran 1968).

3. Results and discussions

The growth in weight of catla and rohu fed on the 4 experimental diets is shown in figures 1 and 2 respectively. From the figures it is seen that the growth pattern of both catla and rohu were more or less similar even though the growth rate of the latter was slower. Rohu is known to grow slower than catla under identical conditions (Alikunhi

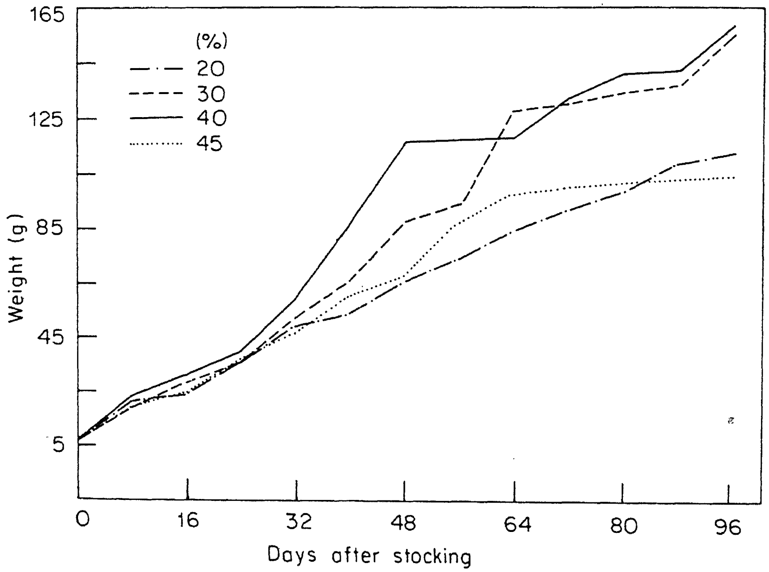

Figure 1. Average weight attained by catla in different treatments.

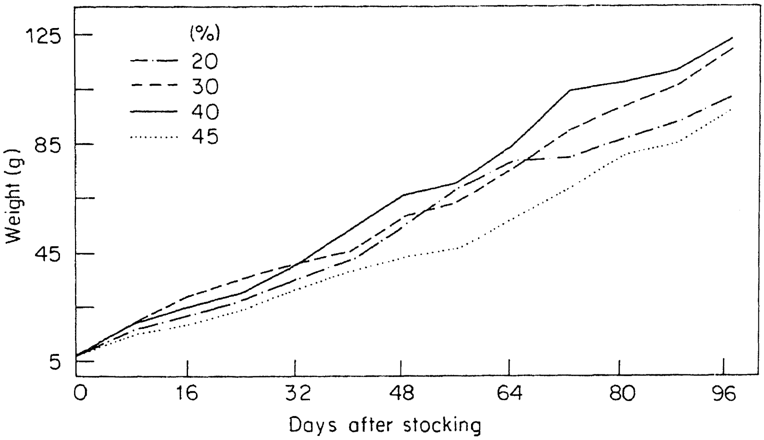

Figure 2. Average weight attained by rohu in different treatments.

1957). The daily average growth rate of catla in terms of weight was the highest and more or less similar when fed on PB containing 30% protein as well as PC containing 40% protein (1·58 and 1·59 g/day respectively), while the growth rate was minimum with PD having 45% protein (1·002 g/day) and slightly better (1·104 g/day) with PA containing 20% protein. In case of rohu, the best growth was recorded when fed on PC followed by PB. As in the case of catla, the difference in growth of rohu when fed with PC and PB was not significant. In rohu also the poorest growth was obtained when fed on PD containing 45% protein, while the growth was slightly better when fed on PA.

Results of the present study revealed that the growth in weight of both catla and rohu was the best and more or less similar when fed on PB and PC, containing 30% and 40% protein respectively. The difference in growth observed between PB and PC was not significant in both the species. Jayaram (1978) reported the best growth of catla and rohu when fed on diets containing 34% and 35% protein respectively. On the other hand, Sen *et al* (1978) observed that the optimum protein level required in diet for spawn, fry and fingerlings of common carp and rohu was 45%. This finding is not in agreement with the results obtained in the present study, where fingerlings were reared over 90 days till they became advanced fingerlings. It is known that the protein requirement of fishes varies with the size (Halver 1976) and water temperature (Delong *et al* 1958). As the fish grow in size, the protein requirement decreases. Therefore, the difference in protein requirement observed by Sen *et al* (1978) and in the present study could be due to the difference in size of the experimental fish. Teshima *et al* (1978) found that the optimum level of protein for *Tilapia zilli* is 30–40%. Jauncey (1982) while working on *Sarotherodon mossambicus*, observed that the optimum protein requirement of the species was 40%. Thus, the protein requirement of *T. zilli* and *S. mossambicus* seems to be greater than that of the Indian major carps studied in the present experiment. The poorest growth of catla and rohu fed on PD containing 45% protein suggest that excess protein content in the diet has an adverse effect on the growth of both the species. The reasons for this growth depression are not understood. Similar observations have been reported by Lim *et al* (1981) in the case of milk fish fed on diets containing higher protein content than the optimum level of 40%. Data on survival rate indicates a better survival of both the species in cisterns where pellets containing 30% and 40% protein are used.

Based on the results of the present study, it can be concluded that both catla and rohu require around 30% dietary protein. Also, it is seen that protein in excess of the optimum level has an adverse effect on the growth of the species investigated. Further, experiments using feeds with only 3–4% variation in protein levels, conducted in flow-through tanks should give more accurate information on the dietary protein requirement of both catla and rohu.

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Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants

R VELAYUDHAN and R S ANNADURAI
Entomology Research Institute, Loyola College, Madras 600 034, India

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Abstract. Pollination by two species of flower thrips in six Solanaceous plants (four perennials and two seasonal) were studied. The results highlight the role of thrips in both self and cross pollination of these flowers. The findings also suggest that in view of the stigmatic surface in all the flowers being at a higher level than the anthers, there is more entomophily. Data relating to the population dynamics of thrips as well as the pollen load of thrips are also included.

Keywords. Thrips; pollination; Solanaceae; pollen load; stigmatic receptivity.

1. Introduction

The pollination potential of thrips though recognised as early as 1914 by Shaw in sugarbeet and by Annand (1926) in such cultivated crops as sugarbeet, alfalfa, plum and daisey, further confirmation of their active involvement in pollination is referable to the contributions of Hagerup (1950) on *Calluna* and *Erica* (Hagerup and Hagerup 1953), Billes (1941) on cacao by *Frankliniella* Odland and Porter (1941) on *Capsicum annuum*, Carlson (1964) on onion and Ananthakrishnan *et al* (1981, 1982 and 1984) on Asteraceae (= Compositae). Except for the work of Odland and Porter (1941) cited above on the role of thrips in the pollination of *Capsicum annuum*, no further information is on record on the other members of Solanaceae. Pollination by thrips and the production of viable seeds in the members of Solanaceae can be correlated with the degree of stigmatic receptivity preceded by the porous dehiscence of the anther, besides the ability of thrips to enter the bud itself. Information presented here highlights the role of thrips in the pollination of *Solanum melongena* Linn, *S. xanthocarpum* Schrad and Wendl, *S. torvum* Swartz, *S. trilobatum* Linn, *S. nigrum* Linn. and *C. frutescens* Linn.

2. Materials and methods

Periodic weekly collections of thrips over one year period (March 1984 to February 1985) were made from 20 flowers of the Solanaceous plants referred to and record kept of the population fluctuations and the pollen load by counting the number of pollen grains attached to various parts of the body. Dispersal of thrips from flower to flower was observed by marking them with gilt powder. A comparative assessment of the efficiency of pollination by various agents was estimated in terms of seed setting on the basis of 3 sets of experiments (i) hand pollination, (ii) pollination by thrips alone and (iii) natural condition where all the other foraging insects played a significant role. The various stages of flower formation have been conveniently grouped into 3 categories viz the bud stage (1–2 days old), the open flower stage (3–4 days old) and the older flower which are on the verge of falling off (5–6 days old), hereafter referred to as stages I, II and III respectively. The receptive areas of the stigma in all the 3 categories of flowers were scanned with the aid of a scanning electron microscope. Investigations on the mode of attachment of pollen grains on the various parts of the body of thrips were made by etherising the specimens carrying pollen grains and dried in a critical point dryer. The specimens were later fixed on to an aluminium stub using a double adhesive tape and was coated with gold with an ion coater and later observed using the scanning electron microscope. Micrographs were taken using Hitachi Scanning Electron Microscope (Model S 415A) under 15 KV emission current.

3. Observation and results

Flowering in both *S. melongena* and *C. frutescens* is restricted to the months of February–May and July–November with both cultivated during the same time in adjacent plots. Such hosts like *S. torvum*, *S. xanthocarpum*, *S. trilobatum* and *S. nigrum* are perennials. Of the two flower infesting species *Frankliniella schultzei* (Trybom) and *Taeniothrips major* Bagnall, the latter is notable for its absence in *C. frutescens* and *S. torvum* while *F. schultzei* appeared to be the dominant species in all the host plants (figures 1 and 2).

Abundance of these anthophilous thrips species is mainly determined by the flowering season of the host plants, availability of their flowers and also the effect of temperature, rainfall and relative humidity (table 1). An optimal temperature (28–32°C), moderate rainfall (upto 50 mm) and high humidity (above 80 %) tend to build-up populations, while heavy rains drastically reduce them. Table 1 also reveals that occasional rains would induce the populations to oscillate.

The continuous availability of different habitable host plants within the same vicinity possibly prevented the complete decline of the population during the rainy season and at the time of non-availability of the flowers in some of these plants. In the case of seasonal crop plants the maximum population was observed between September–October during the first cropping season and in March–April during the second cropping season (figure 2). Population of *F. schultzei* was at its maximum within the flowers of *S. xanthocarpum* (350 ± 8 individuals per 20 flowers) and the least was seen in *S. torvum* (55 ± 2·38 individuals per 20 flowers) among the perennial plants (figure 1). The maximum number of *Taeniothrips major* was observed on *S. trilobatum* (132 ± 2·90 individuals per 20 flowers) and the least in *S. xanthocarpum* (80 ± 1·45 individuals per 20 flowers) (figure 1). A slight preponderance of the population of *T. major* over *F. schultzei* was observed in the flowers of *S. nigrum* and *S. trilobatum* for a very short duration (only in September). In *S. xanthocarpum* the phenomenon of dominance was very evident with *F. schultzei* numbering around 350 ± 8 individuals per 20 flowers while the number of individuals of *T. major* was only of the order of 70 ± 2 individuals per 20 flowers. Among the seasonal crop plants *F. schultzei* was dominant throughout except in the second crop of *S. melongena* where *T. major* dominated in the month of May (figure 2). Intercrop and interfloral migration of thrips

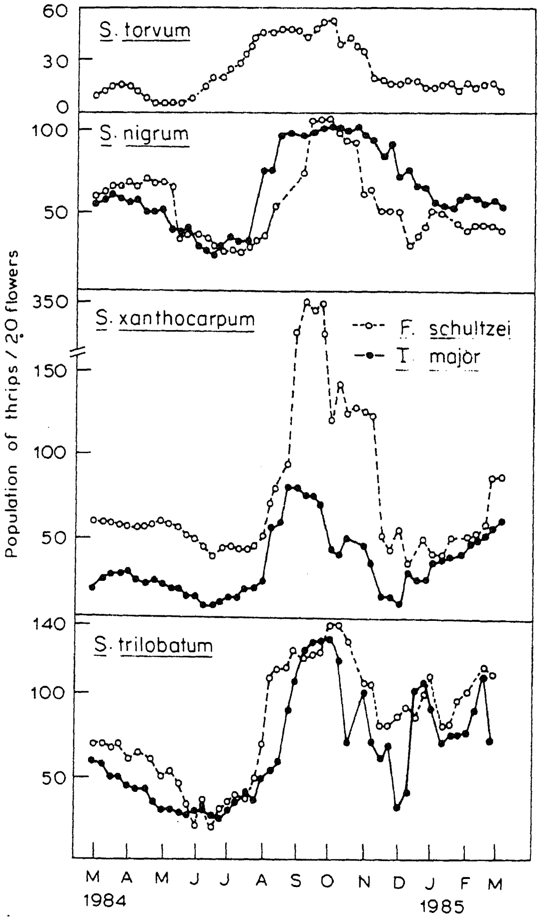

Figure 1. Population dynamics of *F. schultzei* and *T. major* on different perennial solanaceous host plants.

was also occasionally observed, mainly attributable to the type of floral availability which in turn provides shelter and food for sustainance.

3.1 *Pollen load*

A comparison of the pollen carrying capacity of larval and adult *F. schultzei* revealed that the maximum pollen was carried by the larvae than the adults in all the stages of the flower. As against the interfloral movements of adult thrips, the larvae restricted their movement and were found within the flower usually moving between the nectaries and the stigmatic lobe. Besides depicting the maximum and minimum pollen load of adults and larvae of *F. schultzei* (figure 3) the graph also indicate the average pollen load. The maximum pollen load of 79 ± 5 pollen grains was recorded on the body of the larvae in the flowers of *S. melongena* and the least number of pollen grains were found

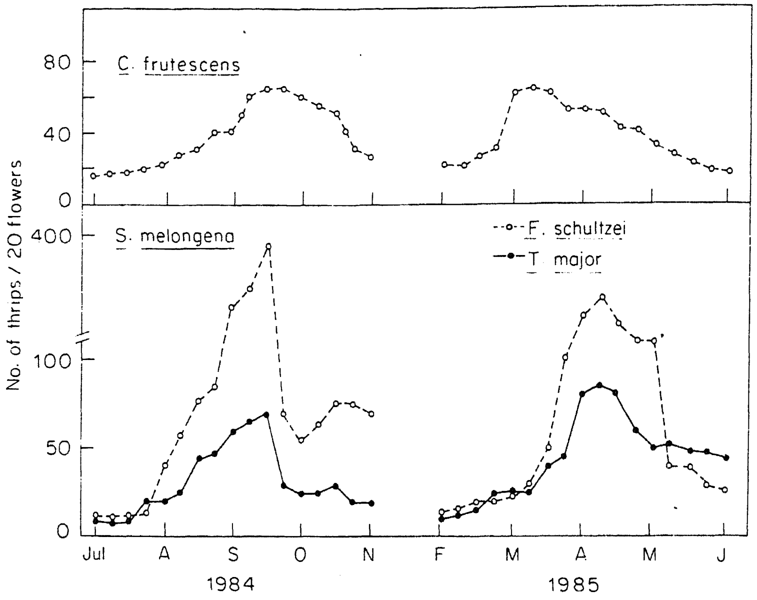

Figure 2. Population dynamics of thrips in seasonal solanaceous crops (*C. frutescens* and *S. melongena*) for two cropping seasons.

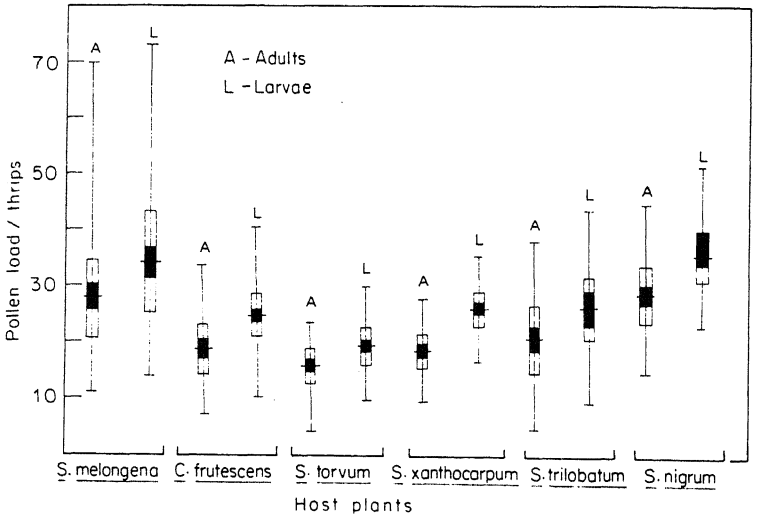

Figure 3. Graph depicting the pollen load of *F. schultzei* (Adults and Larvae) on different solanaceous host plants. The lower peaks indicate the minimum pollen load. The higher peaks indicate the maximum pollen load. The rectangle indicates the standard deviation and the shaded region depicts the standard error. The line bisecting the rectangle indicates the mean pollen load.

Table 1. Population dynamics of thrips along with abiotic factors.

Months	Abiotic factors Average maximum temp °C	Average minimum temp°C	Rainfall mm	Rh (%)	Population of thrips on different hosts *S. xanthocarpum* 1	*S. xanthocarpum* 2	*S. trilobatum* 1	*S. trilobatum* 2	*S. nigrum* 1	*S. nigrum* 2	*S. torvum* 1
March 84	31·07	24·28	0·6	79·16	58·00± 0·40	25·25± 1·90	69·00± 1·00	54·50± 2·56	65·75±0·75	58·50±0·85	13·25±1·38
April	34·23	26·37	1·4	78·95	56·00± 0·70	27·00± 1·75	62·75± 1·30	41·75± 1·97	67·75±1·03	53·50±1·55	10·00±2·20
May	34·79	27·87	—	80·85	50·50± 2·30	19·50± 1·70	44·75± 5·25	28·75± 0·75	51·25±8·85	42·50±3·20	5·75±0·75
June	37·87	27·66	11·7	70·64	46·00± 2·40	11·75± 1·18	26·25± 3·80	28·25± 1·18	32·25±2·28	32·25±2·28	15·75±2·84
July	33·96	24·79	35·4	80·80	44·00± 0·57	17·50± 1·44	40·00± 3·49	35·00± 2·04	28·75±1·49	38·50±5·54	33·25±4·45
August	35·98	26·04	1·5	76·98	76·00± 9·90	55·00±11·33	102·50±10·54	63·25± 9·15	51·25±5·56	85·50±6·08	49·00±0·58
September	33·66	25·62	338·5	81·76	322·50±18·80	75·00± 2·04	122·50± 1·04	126·25± 2·39	93·75±9·52	97·00±1·15	50·00±2·04
October	31·72	24·39	11·7	81·82	127·50± 4·40	45·25± 2·28	128·75± 8·26	101·15±14·50	101·75±1·18	97·25±3·20	44·25±2·39
November	28·27	22·63	273·50	83·20	84·75±22·40	26·25± 7·18	92·50± 7·72	75·00± 8·52	85·25±3·75	55·75±3·87	35·75±5·45
December	28·68	21·22	50·00	81·78	47·25± 3·60	22·50± 4·30	90·00± 3·58	69·25±19·54	68·75±2·39	39·25±4·35	19·00±0·58
January 85	28·32	21·65	51·8	77·92	44·75± 3·30	38·00± 0·91	91·25± 7·68	78·50± 4·17	60·00±2·04	47·50±1·44	16·75±1·18
February	30·27	21·65	—	80·14	78·25± 7·00	50·00± 1·97	108·75± 3·15	86·25± 8·59	68·00±0·72	43·25±1·18	17·75±1·03

1 *Frankliniella schultzei* (Trybom 1910).
2 *Taeniothrips major* Bagnall 1916.
Mean ± Standard error.

on the adults within the flowers of *S. trilobatum* (4 ± 1). The pollen load on the body of *F. schultzei* may range from 13–79 on *S. melongena*, 8–41 on *C. frutescens*, 4–30 on *S. torvum*, 10–36 on *S. nigrum*, 4–45 on *S. trilobatum* and 15–52 on *S. xanthocarpum* and the exact mode of pollen attachment is indicated in figures 4A–C. On the body of the larvae the maximum pollen attachment was observed on the lateral sides of the abdomen and on the last abdominal segments (figure 4C), while in the adults of

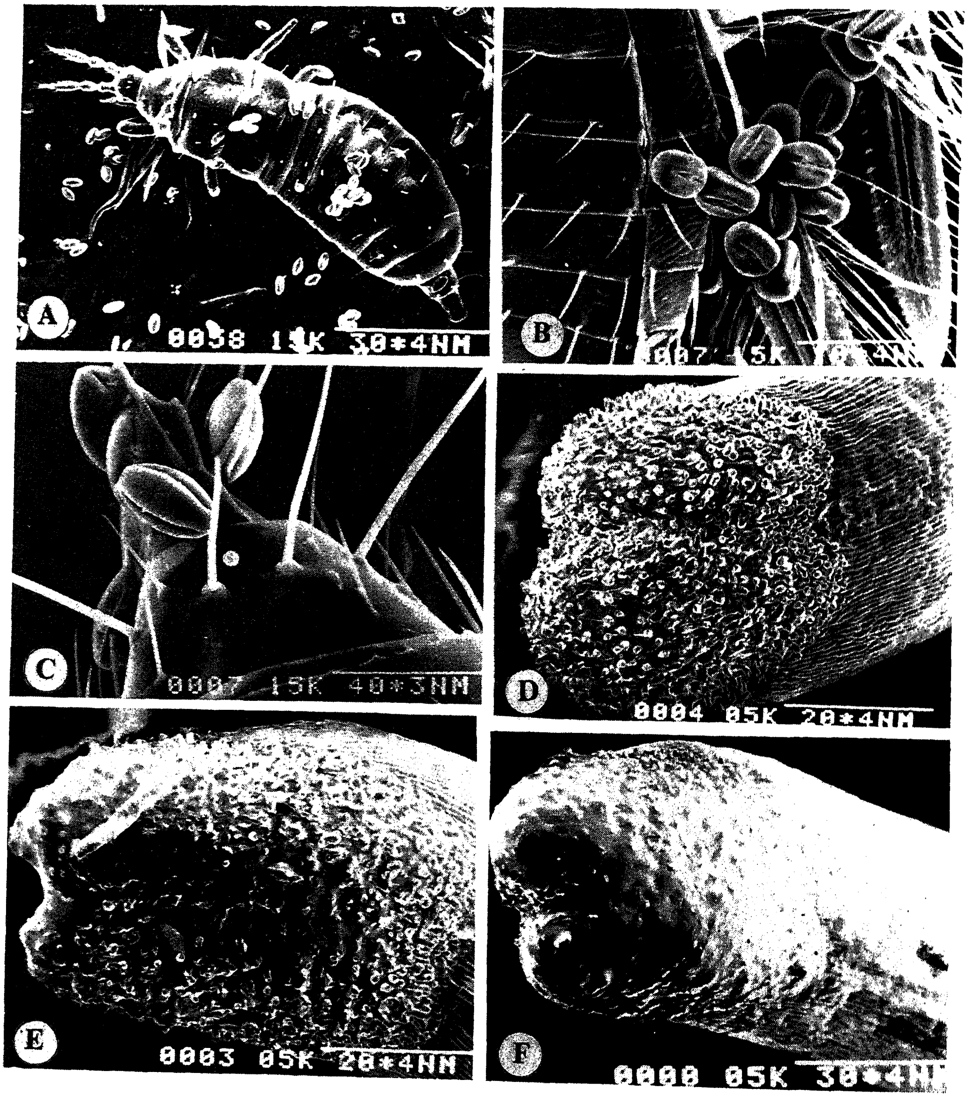

Figure 4. Scanning electron micrographs. **A.** Pollen grains attached to the body of the larva of *F. schultzei*. **B.** Pollen grains attached to thorax and wing fringes of the adults of *F. schultzei*. **C.** Pollen grains attached to the abdomen and the last abdominal segment of *F. schultzei*. **D.** Stage I (bud) stigma of *S. torvum* with closely packed papillae. **E.** Stage II stigma of *S. torvum* with a sticky stigmatic surface and uniformly spread papillae. **F.** Stage III stigma of *S. torvum* with widely spacedout papillae on the dry stigmatic surface.

F. schultzei it was maximum on the thorax and wing fringes (figure 4B).

On the basis of scanning electron micrographs of *S. torvum* (as a representative of the Solanaceous species studied) the nature of the stigmatic surface during the 3 stages of the flower as earlier defined was studied. *S. torvum* flowers are protandrous in nature and the porous dehiscence of the anther occurs between the third and fourth day after the formation of the floral bud. The maturation of gynoecium coincides with the straightening and lengthening of stigma, which by then is at its peak of receptivity and the surface of the stigmatic lobe had evenly spaced out papillae in contrast to the stage I (bud) stigma where the papillae appear to be very crowded (figure 4D). Similarly the stage II stigma seemed to be more sticky than the other two stages probably due to the exudation of the stigmatic surface which incidentally reaches its peak during this stage (figure 4E). The population dynamics of thrips revealed that the maximum number of individuals (both the adults and larvae) were found during the same period. Stage III stigmatic surface showed widely spaced out papillae on its surface and the surface appeared to be completely dry (figure 4F).

The 3 experiments on the comparative assessment of the efficiency of pollination resulted in seed setting of 90 %, 93 % and 97 % respectively. The significant aspect of these experiments was that pollination by thrips alone accounted for 93 % which is comparable with the other two sets of experiments.

It is an accepted concept that in solanaceous flowers if the stigma is of the same length or is shorter than the stamens, then self pollination occurs along with poricidal dehiscence (Vogel 1978). Hence in order to ascertain the above view, the distance between the stamens and the stigma was calculated in all the flowers and in all of them the stigma was located above the stamens and the distance was 1·4 ±0·22 mm in *S. melongena*, 4·55 ±0·20 mm in *S. torvum*, 4·00 ±0·29 mm in *S. xanthocarpum*, 6·00 ±0·20 mm in *S. trilobatum*, 0·20 ±0·017 mm in *S. nigrum* and 1·50 ±0·167 mm in *C. frutescens*. Based on these observation it is concluded that cross pollination or self pollination by the help of insects could be a common phenomenon in the above flowers.

4. Discussion

The coadaptation of the flower and insects involves the mechanism of pollen transfer based on the behavioural patterns of the pollinator. The 'adaptive spectrum' of thrips and the flower focus attention on aspects such as availability of food, stigmatic exudation and other favourable microenvironment within the corolla providing suitable ovipositional site enabling the emerging larvae to be dusted with pollen in their movement from the base of the flower on to the anther or stigma of the flower. The solanum type of 'pollen flowers' (flowers that offer surplus of pollen as reward to their visitor instead of nectar) are all oligandrous with most of their anthers enlarged and showy and also capable of producing excess pollen that are mostly sessile (by neotenic shortness of filaments) and poricidal. The powdery pollen are released in small portions as a 'cloud' and depending on the movement of the pollinator, the various regions of its body are dusted with pollen. Apart from the sticky and viscous pollen kitt which makes the pollen grains adhere, the sticky stigmatic secretions or additional sticky substances further improves the pollen adherence to the insect. The architecture of the pollen wall coupled with the setal arrangements on the body of thrips also aid in pollen attachment to various sites of the body.

Among the various Solanum spp. studied in those species in which the stigma is identical in length as the stamens, self pollination readily occurs. Purseglove (1968) has reported about 16% of cross pollination in *C. frutescens*. In other species in which the stigma projects beyond the anther, it is supposed that pollination is facilitated either by the stigma curving downwards to bring it in line with the falling pollen or by the flower dropping so that the pollen falls on the stigma or perhaps by the aid of insect vectors (Knuth 1908; Stevenson and Clark 1937). Kakizaki (1924 as reported by Free 1970) found that Solanum plants grown in cages without insects produced no fruits. This observation along with the results of the 3 experiments substantiates the view that insect visits appeared necessary for the transfer of the pollen on to the stigma, if the stigmatic receptive surface projects beyond the level of the anthers. The present study also shows that in all the flowers taken for the investigation the stigma is located at a higher level than the anthers, and most probably the transfer of the pollen from the anther to the stigma is mainly done by thrips and especially the larvae which confine their movements only within one flower. On the other hand a slight degree of cross pollination is also effected by the adults making short flights from one flower to another besides being drifted by the wind. Thrips are generally considered to be a homogenous type of pollinator confining to one species of plant and only pollen of that species can be found on the body at a time. Hence this study of solanaceous plants highlights the role of thrips as effective pollinators accounting for both self and cross pollination, with the larvae engaged in a larger percentage of self pollination with the adults occasionally playing the role of cross pollinator.

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Sensillar diversity, density and distribution during post-embryonic development of *Cyrtacanthacris ranacea* Stoll and their role in feeding

T N ANANTHAKRISHNAN, N SURESH KUMAR and
K P SANJAYAN
Entomology Research Institute, Loyola College, Madras 600 034, India

MS received 26 October 1985

Abstract. Diversity, density and distribution of the sensilla of *Cyrtacanthacris ranacea* Stoll during post-embryonic development and their role in feeding are discussed alongside with the scanning electron microscopic studies of these sensilla.

Keywords. *Cyrtacanthacris ranacea*; sensilla; feeding.

1. Introduction

Acridids being generally polyphagous, have been known to show a selective preference for monocots, dicots or both. Since feeding preferences depend on the sensilla that aid in host selection, an understanding of the sensillar nature and distribution is all the more necessary. Blaney *et al* (1971), Blaney (1974) and Chapman and Thomas (1978) provided a basic picture on the nature and distribution of the sensilla on the mouth parts of Acridoidea in general and further studies by Ananthakrishnan *et al* (1985a) indicate that the sensilla on the antenna, maxillary and labial palps, labrum and hypopharynx play a key role in food selection aided by visual host location. Earlier studies on *Cyrtacanthacris ranacea* Stoll, an important pest of cotton relate to food preference in terms of the duration of post-embryonic development, growth rate and quantitative food utilization (Ananthakrishnan *et al* 1985b). Observations presented here relate to the diversity, density and distribution of the sensilla of *C. ranacea* during post-embryonic development and their role in feeding.

2. Material and methods

C. ranacea were collected from cotton tracts and reared in the laboratory on cotton leaves in rearing cages measuring 25 × 25 × 30 cm. Wet soil was provided in a plastic container for the purpose of oviposition. Newly hatched first instar nymphs were segregated and reared in separate cages for various biological studies. For light microscopic observations the labrum, labium, maxillae and antennae of the various stages of the grasshopper were dissected in insect ringer, washed with distilled water, incubated in 1% potassium hydroxide for 5 min, washed well in distilled water and mounted in glycerine. For scanning electron microscopic studies, the mouth parts and the antennae were washed in 30% acetone, and dehydrated in alcohol series, transferred to amylacetate and dried using a Hitachi critical point drier with liquid carbon dioxide. The dried specimens were fixed on aluminium stubs using a double adhesive tape and coated with gold using a standard ion coater for 3 min. Photographs were taken using a Hitachi Scanning Electron Microscope under 15 KV emission current.

3. Observations

3.1 *Sensillar diversity and distribution*

The outer face of the labrum bears a large number of trichoid sensilla along with numerous much longer sensilla most abundant in the central area above the transverse sulcus. The face of the labrum bears a number of campaniform sensilla particularly dense around the edge and along the median groove immediately in front of the mandibles. The inner face of the labrum bears a large number of sensilla of which the most conspicuous are the 4 paired groups of dome shaped sensilla designated as A1, A2, A3 and A10. These 4 groups bear the campaniform sensilla (figure 1G). Light microscopic studies show the epipharyngeal surface of the clypeo-labrum to bear 3 pairs of hair tracts designated as 'α', 'β' and 'γ' and a small group of hairs lateral to the A3 sensilla groups designated as 'δ'. The α-, β- and δ- tracts bear densely packed sensilla trichodea (figures 1 C, D) while the γ-tracts bears the sensilla chaetica (figure 1 E). Detailed observations using scanning electron microscope showed in addition to these 4 tracts, distinct rows of sensilla basiconica arranged in groups of 3–4 (figure 1 H), distributed along the edge of the labrum towards the α-tract, becoming intercepted with a few sensilla chaetica near the α-tract. While the α, β, γ, δ, A1, A2, A3 areas are distinct throughout the post-embryonic development, the A10 group of sensilla are best observed only in the adult stage. Another distinct zone not observed in the nymphal stages, but observed only in the adult is a row of sensilla herein designated as the 'ϕ'-tract. The ϕ-tract is represented by the sensilla basiconica and is seen distributed in the region between the α- and γ-tracts (figure 1 B). The relative positions of the right and left A2 sensilla groups are known to differ in different taxa. In *C. ranacea*, belonging to the subfamily Cyrtacanthacridinae, the two groups are approximately at the same level.

The mandibular sensilla of *C. ranacea* fall into 3 types namely, sensilla trichodea, sensilla chaetica and sensilla without specialised cuticular structures (figures 2 A, B, D). Most of the sensilla chaetica are grouped adjacent to the molar area, while a few occur along the inner edge of this area. The long trichoid sensilla are distributed along the distal inner edge of the mandible (figure 2 D).

Sensilla trichodea are the most common sensilla of the cardo and stipes of the maxilla. The most numerous sensilla of the lacinea are campaniform sensilla, forming a conspicuous group immediately proximal to the hardened apex. In addition, the inner edge is bounded by a row of stout trichoid sensilla and there are a few others scattered over the surface. On the maxillary palps, there are numerous trichoid sensilla scattered on each segment. A group of small trichoid sensilla forms a hair plate at the base of each segment. The dome at the apex of the palp bears a large number of trichoid sensilla (figure 2 E). The labium bears a few trichoid sensilla. The hypopharynx bears extensive tracts of hairs with groups of large trichoid sensilla and campaniform sensilla laterally. The labial palp bears numerous stout trichoid sensilla.

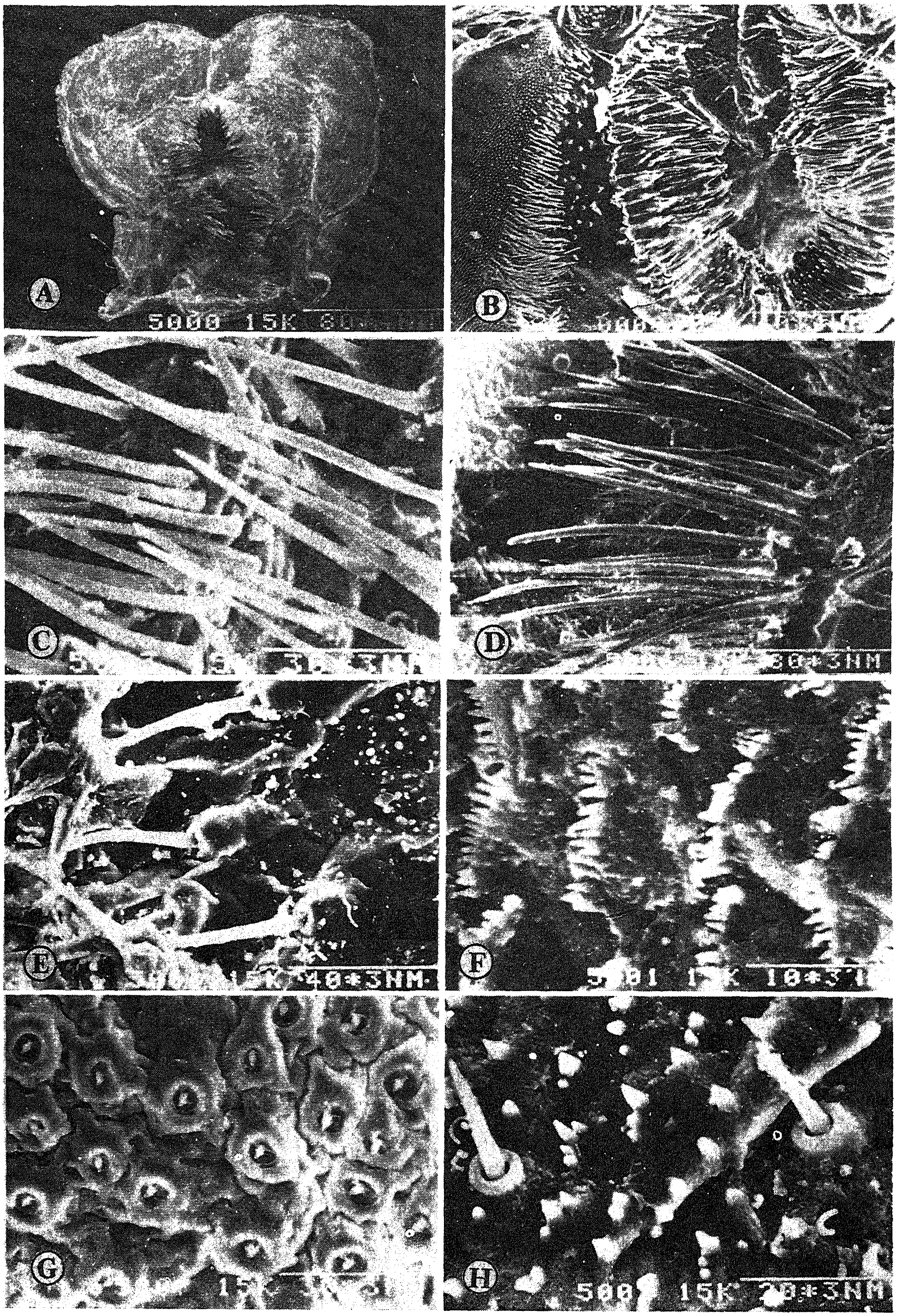

Figure 1. Scanning electron micrographs. **A, B.** Inner side of the labrum. **C,** α-Hair tract. **D.** β-Hair tract. **E.** γ-Hair tract. **F.** Inner edge of the labrum. **G.** A1, A2, A3 and A10 sensilla group. **H.** Between inner edge of the labrum and the α-tract.

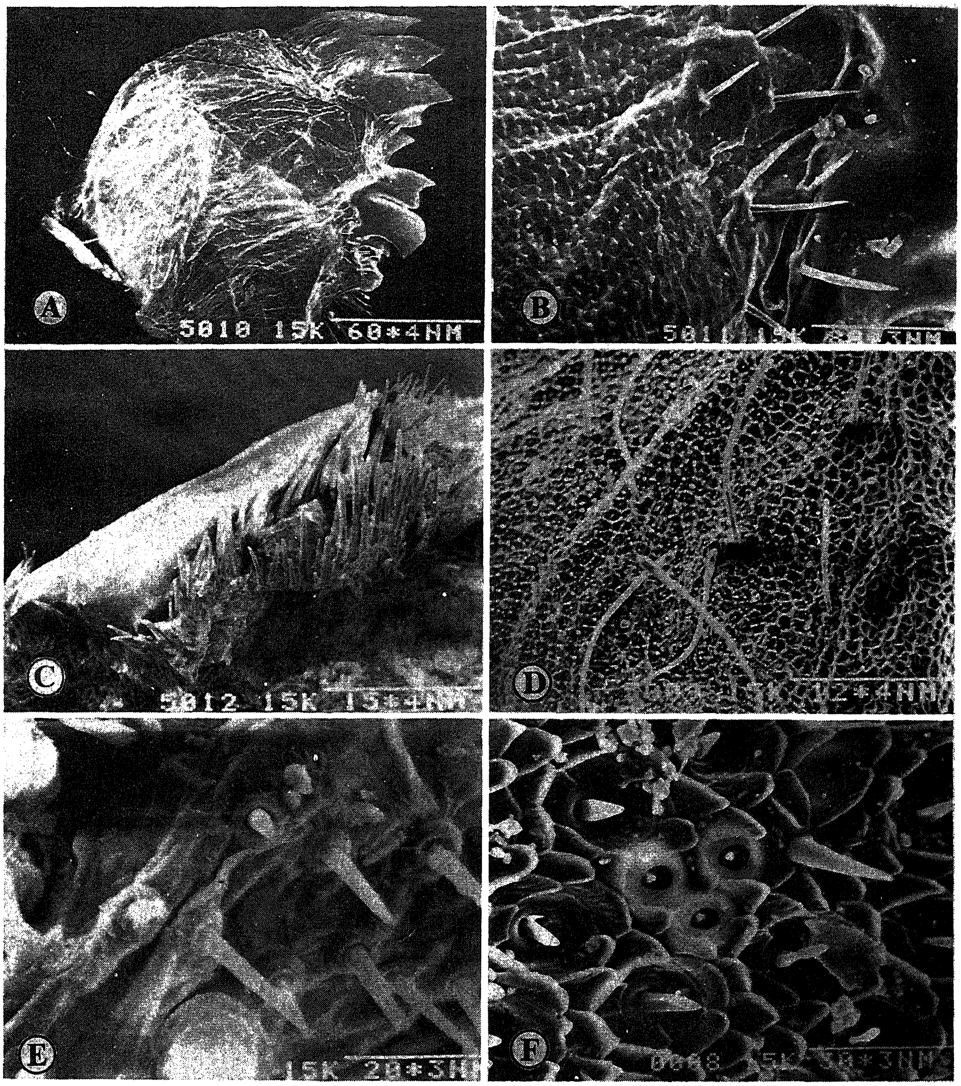

Figure 2. Scanning electron micrographs. **A.** Mandible entire. **B.** Mandible surface inner to teeth. **C.** Hypopharynx. **D.** Lateral surface of the mandible. **E.** Palp tip showing trichoid sensilla. **F.** Antennal surface.

3.2 *Numerical increase in sensilla during post-embryonic development*

An increase in the total number of sensilla of the labrum was evident during post-embryonic development, the rate of increase of each type of sensilla varying considerably. The maximum increase was noticed in the case of the adults, the details of which are evident from figure 3. In addition to the numerical increase in sensillar population during post-embryonic development, distinct differences were observed in the number of these sensilla in each half of the labrum at any particular stage of development. Generally the α- and β-tracts bear more sensilla on the right half than on

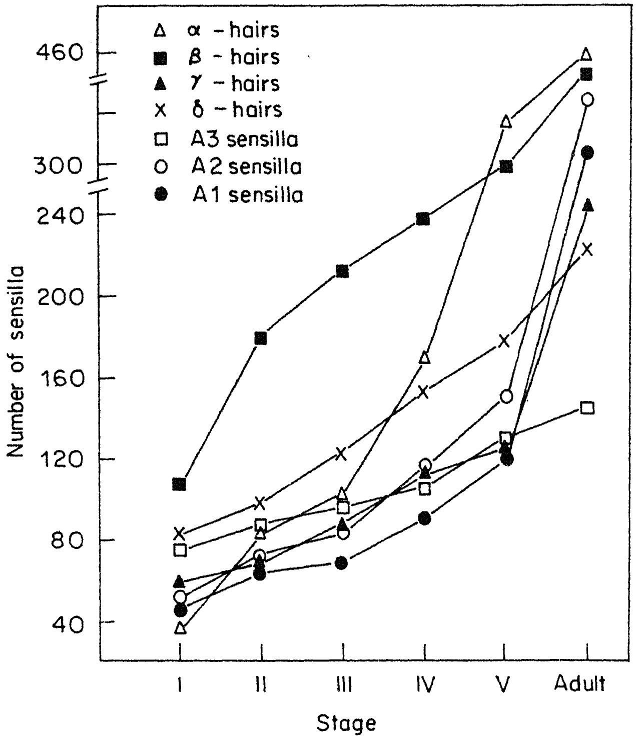

Figure 3. Numerical increase in the various sensilla of the mouthparts of *C. ranacea* during post-embryonic development.

the left while the δ-tract had the sensilla more developed on the left side. Although there were no significant differences in the sensillar count of the left and right sides of the γ-tract during the early nymphal stage, the later nymphal stages and the adult had the sensilla on the left outnumbering the right. Similarly the number of sensilla at the apices of the maxillary and labial palps also increased during post-embryonic development.

3.3 *Role of sensilla in feeding*

Members of the subfamily Cyrtacanthacridinae are generally considered to be mixed feeders. However, extensive field studies showed *C. ranacea* to feed mostly on such malvaceous plants as *Gossypium hirsutum*, *Abelmoschus esculentus*, *Abutilon indicum*, *Sida rhomboidea* etc. Grasses such as *Panicum maximum*, *Cynadon dactylon*, *Cyperus rotandus* did not induce feeding in *C. ranacea*. Feeding in *C. ranacea* as in other phytophagous insects involves sequential behaviour such as host finding, orientation, test bite and feeding. Host finding includes the visual as well as olfactory perception by the sensilla in the antenna. On establishing contact with the host plant, the antennal sensilla as well as the maxillary and labial palps help in food perception leading to the test bite. The movement of the labrum during feeding, facilitates the inward push of the food fragments towards the mouth and the hair tracts further guide the food towards the central core. The densely packed, long β- hairs channelize the food particles towards the A1 and A2 group of sensilla which bear the chemoreceptive campaniform sensilla. The δ- hairs at the tip of the labrum facilitate the movement of the food fragments during test bite towards the A3 sensilla which seem to play a role in initial food selection.

The sensory systems with the different types of sensilla and their receptors, become maximally involved in arriving at suitable food preferences, sampling plants intensively, initially scanning with the sensory apparatus and nibbling, resulting in the intake of small amounts of food, mostly as a result of excitation of the peripheral receptors induced by cues from the plants. Consumption rates and duration of feeding varies with host plants, the degree of consumption being proportional to the time spent on the plant and the quantity of food ingested. Laboratory preference studies for *C. ranacea* from among the malvaceous hosts in terms of the quantity of leaf consumed per day indicate adults of *C. ranacea* to consume 1096·25 mg/day of *G. hirsutum*, 1712·75 mg/day of *A. esculentus* and 1056·25 mg/day of *A. indicum*.

4. Discussion

Studies on the distribution and abundance of diverse sensilla on labrum, maxillary and labial palps of *C. ranacea* indicated significant numerical and distributional variations besides recognition of further types of sensilla in the inner face of the labrum. In addition to the usual α-, β- and γ-tracts, additional δ- and ϕ-tracts were evident. This increase in the types of tracts may be presumed to be essential for accurate perception of various stresses. In Acridids, the number of sensilla increases with the size of the insect. The scattered sensilla have the highest rate of increase, but the number per unit area decreases as the insect get bigger, reflecting progressively wider spacing in the bigger insects (Chapman and Thomas 1978). The apical sensilla of the palps have a higher rate of increase than those elsewhere on the mouth parts. The increase in sensilla at the apices of the maxillary and labial palps of *C. ranacea* during post-embryonic development is a feature also observed by Ananthakrishnan *et al* (1985a). However, in *C. ranacea* trends for an increase in sensillar number were recognized even from the first instar nymph onwards, such an increase can be correlated with the increase in the quantitative food intake as well as the range of preference for their host plants. The relative positions of A2 sensilla between the right and left sides in *C. ranacea* is a feature also observed in *C. tatarica* (L) and *C. aeruginosa* (Stoll).

Food selection in grasshoppers is known to depend, besides a number of physical and chemical factors, on the stress conditions of the insect since an oligophagous grasshopper will eat a wider range of plants under severe conditions of stress (Bernays *et al* 1976). In general, grasshoppers are classified into those that feed on grasses or on broad-leaved plants or on a mixture of the two. The Pyrgomorphidae and Catantopinae mostly feed on broad-leaved plants, while the Hemiacridinae, Acridinae, Oedipodinae and Gomphocerinae feed most exclusively on grasses. Some members of the subfamilies Eyprepocnemidinae, Melanoplinae, Cyrtacanthacridinae and Oxyinae are mixed feeders in the sense that they feed on either grasses or forbs. *C. ranacea* is a restricted oligophagous grasshopper feeding mainly on plants belonging to the family Malvaceae. The present study indicates that *C. ranacea* is specific to feeding on malvaceous plants. Although reports of food specificity in Acridoidea appear meagre, *Anablepia granulata* has been shown to feed only on *Brachyaria* spp (Gillon 1972); *Cornops longicorne* to be associated with *Eichhornia* (Perkins 1973) and *Poekilocerus hieroglyphicus* to be associated with Asclepiadaceae (Abushama 1968). In all these insects, it was shown that the number of sensilla is comparatively less than the members of their related group. Chapman and Thomas (1978) opined insect specificity to be associated with the development of the sensilla turned to key chemicals in the normal food plant; such a development would permit a reduction in the numbers of sensilla involved in host selection. However, it does not follow that a limited host plant range will always be associated with a relatively small number of sensilla.

The sensory mechanism underlying feeding behaviour in Acridids is a complex one. Haskell and Schoonhoven (1969), Cook (1972), Chapman and Thomas (1978) and Chapman (1982) have indicated the chemosensory nature of the A1, A2 and A3 sensilla and suggested others probably as mechanoreceptors. Studies by Blaney (1974) and Haskell and Schoonhoven (1969) suggested the mechanical and chemosensory nature of the sensilla on the surface of the maxillary and labial palps, which are known to increase as they grow into adults. Although the function of the hair tracts along the inner side of the labrum is not known for certain, present observations on the movement of the food and the role of sensilla are further supported by the observations of Cook (1977) who showed that fluids spread more rapidly along these tracts than over the general surface of the epipharynx. Large amount of information on all qualities of food, both phagostimulatory and deterrent, are fed into the central nervous system of the insect and the final motor behaviour comes about as a result of filtering and integration in higher centres (Haskell and Mordue 1970), an aspect needing further investigation.

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Composition and variations in the abundance of zooplankton in the Hooghly estuary, West Bengal, India

S K SARKAR, B N SINGH and A CHOUDHURY

S D Marine Biological Research Institute, Sagar Island, 24-Parganas and Department of Marine Science, Calcutta University, Calcutta 700019, India

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Abstract. Total zooplankton biomass and the constituent species under the major taxa in relation to hydrographic conditions in the Hooghly estuarine complex were studied around Sagar Island during March 1979 to February 1981. Higher numerical counts and biomass of zooplankton occurred during high saline premonsoon period. During monsoon efflux most of the stenohaline organisms disappeared and only the low salinity species alongwith the euryhaline ones were able to thrive in the estuary. Copepods constituted the most predominant portion of zooplankton biomass throughout the year. Two peaks of zooplankton were invariably noticed—primary major peak during June/July and January/February showed the secondary major peak.

Keywords. Zooplankton; composition; biomass; Hooghly estuary; West Bengal.

1. Introduction

A correlative study on zooplankton distribution, their intensity and seasonal abundance in relation to environmental parameters is necessary for a correct understanding of the fishery resources. Information on zooplankton in a vast tropical estuary like that of the river Hooghly are rather inadequate (Dutta *et al* 1954; Shetty *et al* 1961; Saha *et al* 1975; Bhunia 1979; Bhunia and Choudhury 1981; Sarkar *et al* 1984a,b; 1985 a,b,c,d). The present investigation intends to document the nature, composition, distribution and variations of the surface zooplankton in the lower stretch of the Hooghly estuary.

2. Material and methods

2.1 *Area investigated*

Sagar Island, the western most part of Sunderbans in the Bay of Bengal, is situated 96 km south of Calcutta, between latitudes 21°37′ and 21°52′N and longitudes 88°03′ and 88°11′ E. It is surrounded by large bodies of water, the river Hooghly in the north and the north-western side and river Mooriganga in the eastern side. The southern part of the island faces the open sea, Bay of Bengal. For convenience of study, 3 areas around Sagar Island were selected for the present investigation—Kachubaria (station 1), Chemagari creek (station 2) and south Sagar (station 3) (figure 1).

2.2 *Techniques of study*

Surface zooplankton samples were collected fortnightly from the 3 stations during high tides in the forenoon hours. Measured quantities of surface water samples were

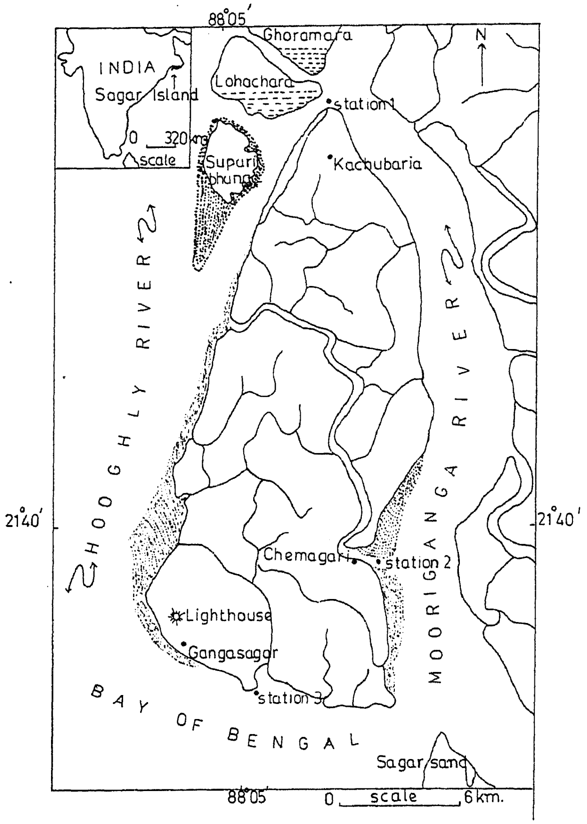

Figure 1. Sagar Island, showing the study areas.

filtered through a conical net (0·25 m diameter and 0·0695 mm aperture) and preserved in 4 % buffered formaldehyde in seawater. In the laboratory, aliquot sample were taken in Sedgewick Rafter counting cell under a compound microscope for different numerical analyses. From each plankton collection, 3 estimations were made and the mean was recorded in no./m^3. Water samples were taken simultaneously for the determination of various hydrological parameters and were analysed by the methods of Strickland and Parsons (1968). Water transparency was determined with the help of a secchi disc and precipitation data were collected from the Alipure Meteorological Observatory, Calcutta. For estimating the plankton volume, the displacement method was used.

3. Results and discussion

3.1 *Hydrology*

Hydrology of Hooghly estuary, presents a cyclic pattern, characterized by large amount of precipitation and tidal interplay (Sarkar *et al* 1985d). A marked difference in the hydrological parameters in different seasons was noticed at all the stations. During the course of study, the water temperature was higher in the premonsoon months

(March–June) and lower during the postmonsoon months (July–October). Variations in surface water temperature were normal for a tropical estuary with maximum value in June (31·8°C at station 3) and minimum during January/February (21·0°C at stations 1 and 3). Every year the salinity gradient increased to a maximum during late premonsoon months at all the stations paralleling with the higher temperature and decreased during the monsoon months and was inversely proportional to the rate of rainfall. The oscillatory fluctuations were very much pronounced at station 1 as the strong freshwater flow from upper reaches of the Hooghly estuary dominates the high saline seawater. The maximum and minimum values recorded were 23·77 and 1·87 ‰ at station 1, 25·84 and 3·12 ‰ at station 2 and 31·31 and 7·76 ‰ at station 3, respectively. Seasonal variations in the dissolved oxygen values were observed but not conspicuous as salinity. The maximum value was recorded during monsoon and postmonsoon months and minimum in summer season. pH did not show major fluctuations but varied during monsoon months. Secchi disc readings indicated least values in turbidity during the late monsoon months. Owing to soil erosion and admixture of water, station 3 experienced high turbidity all through the year.

3.2 *Biomass value*

The zooplankton standing crop as determined by biomass was high during the premonsoon period, low during late monsoon months and with relatively minor secondary value during the postmonsoon season (figure 2). Maximum displacement volume (1·04 ml/m^3) was recorded in June 1980 at station 2. Due to greater abundance of megalopa present in the sample in February 1981, the biomass showed a higher value in comparison with the numerical value of total fauna.

3.3 *Composition of zooplankton*

Spatial and temporal distribution of zooplankton showed definite seasonal trends associated with environmental fluctuations (figure 3). These include copepod, mysid,

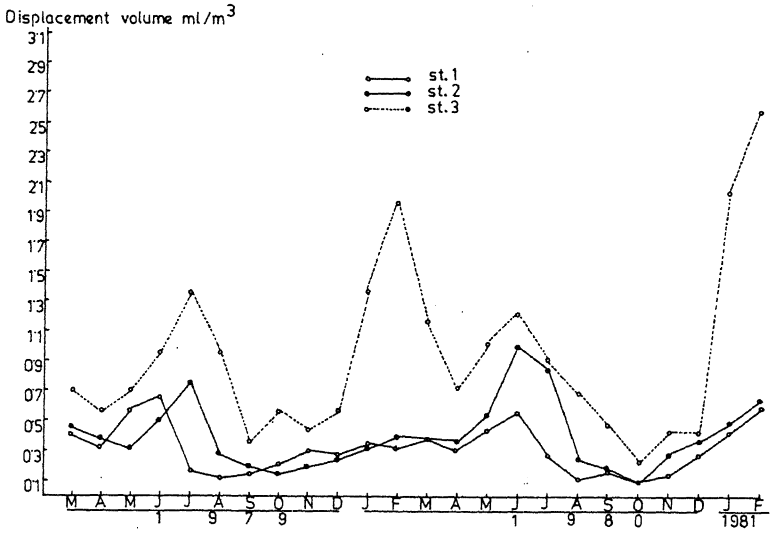

Figure 2. Monthly average biomass values at stations 1, 2 and 3.

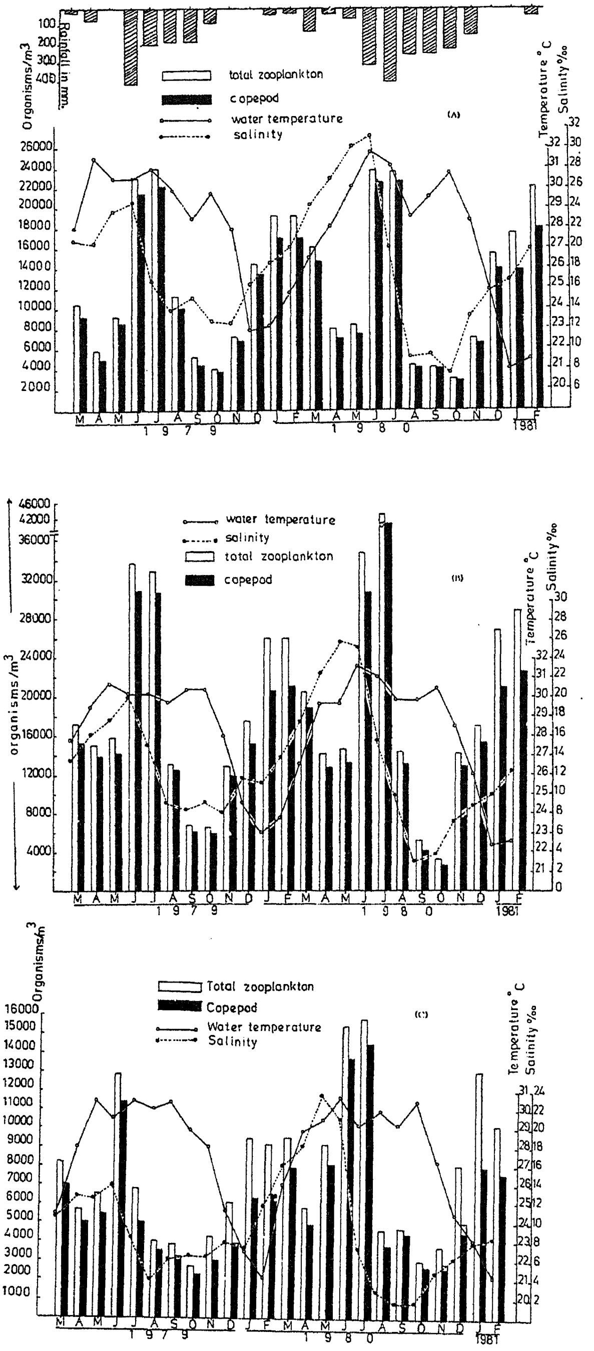

Figure 3. Seasonal trends in the density of total zooplankton and copepod in relation to other environmental parameters (A) station 1 (B) station 2 and (C) station 3.

lucifer, gammarid amphipod, cladoceran, ostracod, cumacea, hydromedusae and chaetognath among holoplankters. Qualitative and quantitative variations in the faunistic compositions were noticeable during different months. The most striking feature in the zooplankton abundance in the Hooghly estuarine system was the contrast between the premonsoon and late monsoon periods. Some of the important zooplankters and their species are given below.

I. *Holoplankters*

(A) *Crustacea*

(a) *Copepoda*: Copepods were the most dominant groups of the plankton community throughout the year at all the stations constituting 73·0–96·4% of the total zooplankton. They formed the bulk of the zooplankton biomass. The pattern of bimodal type of copepod distribution closely followed that of biomass with relatively higher values in June/July, a gradual trend of rise in January/February. A sharp fall in copepod population associated with the lower diversity of copepod species were observed in late monsoon months. Out of 32 genera and 54 species recorded from the studied area, calanoid copepods were represented by 17 genera and 37 species, cyclopoids by 7 genera and 9 species and harpacticoid copepods by 8 genera each having single species. During monsoon months, a good assemblage of oligohaline species e.g., *Acartiella keralensis, Neodiaptomus strigilipes, Pseudodiaptomus binghami, Halicyclops tenuispina, Mesocyclops* sp., and *Cyclops* sp., were encountered especially at station 1 for the greater inflow of freshwater from the upper stretch of Hooghly river. These copepod species were totally absent in other two stations. Copepods can be divided into 3 distinct categories depending on their period of existence in the study area. The first comprises the euryhaline species which are present almost throughout the year in considerable numbers. The second group is comprised of those species which have a limited period of existence depending on the variable salinity. The species which are casual migrants constitute the third category. Species composition of the 3 categories are listed below:

Category I

Paracalanus sp., *Acrocalanus* sp., *Acartia* sp., *A. spinicauda, Labidocera euchaeta, Pontella andersoni, Eucalanus subcrassus, E. elongatus, Pseudodiaptomus annandalei. P. hickmani, Oithona* sp., "*Saphirella*" cf. *indica, Microsetella rosea, Laophonte* sp.

Category II

Pseudodiaptomus aurivilli, Euchaeta marina, E. wolfendeni, Euchaeta sp., *Centropages dorsispinatus, Acartiella sewelli, Corycaeus danae, Euterpina acutifrons, Cladorostrata brevipoda, Harpacticus* sp., *Clytemnestra scutellata, Macrosetella gracilis.*

Category III

Acartiella keralensis, Labidocera sp., *L. minuta, Pontellopsis herdmani, Eucalanus* sp., *Pseudodiaptomus tollingeri, P. masoni, P. binghami, P. daughlishi, Pseudodiaptomus* spp., *Neodiaptomus strigilipes, Euchaeta tenuis, E. concinna, Centropages furcatus, Temora turbinata, T. discaudata, Tortanus gracilis, T. forcipatus, Candacia bradyi, Canthocalanus pauper, Undinula darwini, Corycaeus catus, C. agilis, Oncaea venusta, Halicyclops tenuispina, Mesocyclops* sp., *Cyclops* sp., *Tachidius discipes.*

(b) *Mysidacea:* An euryhaline species of this group-*Mesopodopsis orientalis* Tatersall, was collected almost throughout the study period at all the stations. It exhibited almost uniform abundance with little increase in pre- and postmonsoon periods. Maximum density (50 no./m^3) was recorded in January 1982 at station 3.
(c) *Sergestidae:* The presence of this group, being represented by single species, *Lucifer hanseni* Nobili, was noted in monsoon and postmonsoon months. During late monsoon months, this species showed a maximum abundance of 80 no./m^3 at station 2 in September 1980.
(d) *Amphipoda:* Gammarid amphipods were present in pre- and postmonsoon periods and showed variations between 4–28 no./m^3. No definite pattern in their distribution could be outlined since many gammarid species could not be identified.
(e), (f) *Cladocera and Ostracoda:* These two groups were represented by the genera *Evadne* spp., and *Philomedes* sp., respectively and were present in small numbers during high saline period, the latter being totally absent at station 1.
(g) *Cumacea:* Cumacea, constituted by single species *Paradiatylis* sp., was sporadically present in the premonsoon months at stations 2 and 3 with the maximum average density of 135 no./m^3 in June 1980 at station 2.

(B) *Non-crustaceans*

(a) *Chaetognatha:* Chaetognath was represented by two species, *Sagitta bedoti* Beraneck and *S. enflata* Grassi, the former being dominant throughout the year, tolerating a wide salinity range (1·87–31·33 ‰). Sporadic occurrence of *S. enflata* was encountered during relatively high saline period (13·31–31·34 ‰) at stations 2 and 3.

(b) *Hydromedusae:* This group was present in meagre amount during high saline premonsoon period with an average density of 4–18 no./m^3.

II. *Meroplankters*

(a) *Polychaete larvae:* This group appeared in considerable numbers throughtout the year being absent or poorly represented during the late monsoon period followed by a gradual trend of increase during postmonsoon period.

(b) *Crustacean larvae:* Nauplius and zoea conceivably represented the bulk of crustacean larvae followed by megalopa, decapod larvae (e.g., *Penaeus monodon, P. indicus, Metapenaeus monoceros, M. dobsoni, M. brevicornis, M. affinis* and megalopa larva), anomuran larva and alima larva. The last two larval stages were present only at station 3 in small numbers.

(c) *Fish eggs and larvae:* Various groups of fish eggs and larvae were present during certain months of the year.

3.4 *Statistical analysis*

Simple, partial and multiple correlation coefficients between the population density of *S. bedoti* and two environmental parameters (water temperature and salinity) at 3 stations have been shown in table 1. The positive correlation between temperature and salinity indicates that the changes in one are associated with the other and that these two factors together explain a great deal of variation that occurs in the zooplankton composition in the estuary. Again, a positive and highly significant partial correlation between *S. bedoti* and salinity, eliminating the effect of water temperature, suggests that chaetognath population is greatly influenced by the salinity gradients of the sourrounding medium. Temperature and salinity give a positive and highly significant multiple correlation coefficient with the chaetognath, suggesting that temperature and salinity have considerable influence on chaetognath population and a great deal of variation of chaetognath population is caused by these two parameters.

Table 2 represents the statistical correlation coefficient (r) values between common

Table 1. Simple, partial and multiple correlation coefficients between population density of *Sagitta bedoti* (M), salinity (Y) and water temperature (Z) at stations 1, 2 and 3.

	r_{MY}	r_{MZ}	r_{YZ}	$r_{MY\cdot Z}$	$r_{MZ\cdot Y}$	$R_{M\cdot YZ}$
Station 1	0·7325[b]	−0·1231	0·0530	0·7458[b]	−0·2382	0·7501[b]
Station 2	0·4434[a]	0·2882	0·1959	0·4120[a]	0·2290	0·4887[a]
Station 3	0·6119[b]	−0·3090	0·1091	0·3424	−0·4778[a]	0·5172[b]

[a] Significant at 5% level. [b] Significant at 1% level

Table 2. Showing relationship between zooplankton population and hydrological parameters at station 2.

	Correlation values (r)				
Zooplankton	Water temperature	Salinity	Dissolved oxygen	pH	Transparency
Paracalanus spp.	−0·0851	0·4904[b]	−0·3441	0·6012[b]	0·4273[a]
Acrocalanus spp.	−0·0065	0·4637[a]	−0·3667	0·6643[b]	0·4953[b]
Acartia sp.,	−0·8495[b]	−0·3150	0·1069	0·0947	0·3460[a]
Acartiella sewelli	0·2526	−0·7200[b]	0·7246[b]	−0·7672[b]	−0·4028[a]
Labidocera euchaeta	0·0074	0·4752[b]	−0·4329[a]	0·6018[b]	0·5204[b]
Eucalanus subcrassus	0·1092	0·7647[a]	−0·6283[b]	0·4790[b]	−0·0124
Pseudodiaptomus annandalei	−0·3334	−0·2257	0·1316	0·0648	0·0064
Oithona sp.,	−0·2160	0·4802[b]	−0·4034[a]	0·6549[b]	0·5954[b]
Saphirella indica	0·2309	0·8892[b]	−0·5628[b]	0·5410[b]	0·1437
Microsetella rosea	0·4039[a]	0·6671[b]	−0·3625[a]	0·4509[b]	0·1616
Lucifer hanseni	0·4328[a]	−0·3206	0·5062[b]	−0·4639[b]	−0·3657[a]
Hydromedusae	0·4464[a]	0·7833[b]	−0·5076[b]	0·5404[b]	0·0474
Amphipoda	−0·0152	0·7098[b]	−0·4523[a]	0·5096[b]	0·3472[a]
Mysid	−0·0772	−0·2033	−0·1749	−0·1384	−0·0213
Polychaete larvae	0·1878	0·3892[a]	−0·0903	0·3806[a]	0·3333
Nauplius	−0·8195[b]	0·0736	−0·1683	0·4462[a]	0·5696[b]
Zoea	−0·7614[b]	0·1462	−0·2150	0·4973[b]	0·5184[b]
Megalopa	−0·6470[b]	0·0092	−0·0142	0·3318	0·2881

[a] Significant at 5% level. [b] Significant at 1% level.

zooplankton species with the hydrological parameters at Chemagari creek. Significant correlation coefficient (*r*) values have been obtained among some common zooplankton species, salinity and pH. Correlation values between zooplankton species and water temperature indicated that most holoplankters showed no significant values while meroplankters showed negative correlation with water temperature paralleling the abundance of meroplankters during low temperature regime of post-monsoonal period. Again, almost all meroplankters showed no significant values with salinity and dissolved oxygen. Zooplankton species, as a whole, showed no significant values with transparency values indicated that the parameter had almost no effect on their seasonal population fluctuations.

The organisms inhabiting the estuary can be expected to react and adapt to physical, chemical and biological changes of the environment. Being a tropical estuary, temperature fluctuations were not significant enough to inhibit the recruitment of these organisms. Food supply seldom acts as a limiting factor in a tropical estuary and does not seem to govern the seasonal abundance (Qasim 1970). The fluctuation range of dissolved oxygen and pH at the surface waters in the Hooghly estuary are little to moderate. Hence salinity, with wide range of fluctuations, appears to be the major hydrographic factor controlling the incidence of these organisms which has been endorsed by other workers (Chandramohan 1977; Madhupratap 1980; Gajbhiye *et al* 1981; Goswami 1982). A seasonal pattern in distribution reflecting the seasonal change in salinity was exhibited by all groups of zooplankton.

Monsoon flows exert profound influence on every aspect of estuarine hydrobiology and zooplankton. Considering the importance of the effects of monsoon in the system, Hooghly estuary may be called as a 'tropical monsoon estuary'. Rapid fall of salinity, consequent on heavy precipitation and surface drainage interferes with the distribution of marine forms, leading to conspicuous reduction in population leaving a few euryhaline and oligohaline species.

An intermediate condition prevails during the postmonsoon period permitting rapid multiplication of the brackishwater forms and reassemblage of the groups which prefer salinity and temperature, thereby ensuring sufficient recruitment of population in the estuarine waters.

Copepods showed the highest numerical dominance among the zooplankton groups. The pattern of succession gathered from the dominance broadly outlined 3 species categories. They are the high saline species, low saline species and species preferring medium salinities. The highest (31) and the lowest (8) number of copepod species in this estuarine system were obtained during premonsoon and monsoon seasons, respectively. The presence of only a few copepod species during the monsoon months has also been reported from the other Indian estuaries (Pillai *et al* 1973; Madhupratap 1980; Goswami 1982). Apart from salinity, strong currents and transparency may also render the environment 'severe' for their existence.

Although carnivores such as hydromedusae and chaetognath thrived in the estuary only during the high saline period when a higher standing stock of zooplankton was present, salinity rather than food seems to limit their presence during the monsoonal period.

Abundance of decapod larvae and the occurrence of fish eggs and larvae almost throughout the year accentuates the importance of the estuary as the breeding and nursery ground for these groups.

From the available data it appears that the annual zooplankton distribution in

Indian estuaries shows bimodality with two peak periods and it varies from region to region. In the north Kanara coast of India it has been observed that the production of zooplankton shows the maximum peak in August–November which coincided with the diatom peak (Ramamurthy 1965). In the Bombay coast, the volume of total plankton showed maximum peaks in March/April and October/November (Pillai 1968; Radhakrishna and Pillai 1978). Raghunathan and Srinivasan (1983) from Ennore estuary, Madras also noted two peaks during December and February and calanoid dominance in plankton production. Prasad (1954) observed the higher standing crop of zooplankton in March in Palk Bay, and lower peak in September–October. Subbaraju and Krishnamurthy (1972) found the abundance in April and September at Porto Novo waters. In the Hooghly estuary, particularly at the upper stretch of the river, Dutta *et al* (1954) recorded two peaks, one in October–January and the other in June–July. Shetty *et al* (1961) also reported two peaks, one in November–February and the other in June–August. In the lower stretch of the estuary, Bhuina and Choudhury (1981) and Baidya and Choudhury (1984) recorded two peaks, one in March–April and the other in November–December. The present observation also corroborates the preceding documents.

Dominance expresses the magnitude of influence exerted by a species on its habitat and thus is not only dependent on the number of individuals but also their biomass (Debauche 1962). Copepods, no doubt, showed highest numerical dominance and contributed to the bulk of the standing crop. But apart from the numerical dominance, the ecological dominance exerted by other groups, especially carnivores such as hydromedusae and chaetognath cannot be overlooked. These being larger organisms formed a significant portion of the biomass eventhough occurring in smaller numbers. Their utility, whether positive or negative since they are carnivorous in the energy transfer in the trophic system, is also of considerable importance.

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Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera)

G K MANNA and S K DEY
Department of Zoology, University of Kalyani, Kalyani 741 235, India

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Abstract. Male largid bugs, *Physopelta schlanbuschi*, having $2n = 17$ chromosomes (12 autosomes $+ 2m + X_1X_2Y$), were irradiated with x-ray doses of 300 r, 400 r and 500 r which yielded various types of chromosome aberrations in different stages of meiosis of which the common forms were breaks, fragment of unknown origin, constriction, gap etc. Among the 3 sex chromosomes, the two conspicuously large markers, X_1 and Y, sometimes formed chiasma-like configuration in prophase I and metaphase I, while a number of anaphase I plates had a chromatid bridge, very likely formed by the X_1 and the Y. The qualitative and quantitative assessments of chromosome aberrations in spermatogonial metaphase, prophase I, metaphase I, anaphase I and metaphase II were made at 13 intervals for the doses of 300 r and 400 r and 14 intervals for 500 r between 5 min and 1 week or more. The data showed over-all dose-dependent aberration effects and the sex chromosomes appeared relatively more vulnerable than the autosomes to different doses of x-rays. The testes of untreated males taken as controls had practically no aberration.

Keywords. X-ray effects; meiotic chromosomes; *Physopelta schlanbuschi*

1. Introduction

The effects of ionizing radiations have been extensively studied among insects having chromosomes with localized centromere like grasshoppers (Ray-Chaudhuri 1961; Manna and Mazumder 1962, 1967, 1968; Mazumder and Manna 1967, 1969; Fox 1967; Westerman 1968; Kumaraswamy and Rajasekharasetty 1975) and *Drosophila* (Kvelland 1962; Lea 1962; Becker 1975; Falk and Jacoby 1975). These relate to verification of the target hypothesis, mechanism of structural rearrangements, multiple configuration at meiotic stages, intra- and interspecific radiosensitivity, radioprotection etc. On the other hand, the hemipteran species claiming to have a diffuse centromeric activity (Scharader 1953) were deployed by different workers mainly to verify the centromeric constitution of chromosomes (Hughes-Schrader and Ris 1941; Hughes-Schrader and Schrader 1961; Halkka 1965; La Chance and Degruillier 1969) while Manna and his collaborators (Manna 1984; Manna and Dey 1981, 1983; Barik and Manna 1981; Dey and Manna 1983, 1984) have conducted a series of experimental studies on the x-ray induced chromosome aberrations in some species of Heteroptera, not only to verify the centromeric problem, but also to assess the differential radiosensitivity of chromosomes, localization of breaks, induction of pseudochiasma in sex chromosomes, radioprotection by penicillin etc. In the present paper, the qualitative and quantitative effects of 3 doses of x-rays on chromosomes at different stages of meiosis in the males of *Physopelta schlanbuschi* have been dealt with in regard to the dose-dependent response, differential radiosensitivity between and within chromosomes, etc.

2. Material and method

The genus *Physopelta* has been cytologically very interesting because of the sex chromosomal polymorphisms in its different species (Manna *et al* 1985). For the present study, adult males of *P. schlanbuschi* (Largidae: Heteroptera) were irradiated with 300 r, 400 r and 500 r by the x-ray apparatus fitted with the thermax therapy tube, operated at 110 KV, 4 mA, using 1 mm thick aluminium filter. The testes of the irradiated males were fixed in acetic-alcohol (1:3) and each testis was squashed in 45 % acetic acid on a clean slide smeared with Mayer's albumin. The squashed material on the slide was stained in iron-alum haematoxylin, dehydrated in absolute alcohol passing through up-grades, cleared in xylol and mounted in Canada balsam. The untreated males were kept in the same laboratory condition, the testes of which served as control. Assessment of the irradiation effect in different stages of meiosis could not be made from a uniform number of cells because of the rarity of some stages. The spermatogonial metaphase complement of 17 chromosomes consisted of 12 medium sized autosomes, 2 very minute *m*-chromosomes and 3 sex chromosomes (X_1X_2Y), which showed a typical heteropteran post-reductional meiosis (Manna 1951; Ray-Chaudhuri and Manna 1955). In the spermatogonial complement, the X_1 and the Y were of the same size, representing 2 largest marker chromosomes, the X_2 was of the medium size, indistinguishable from the 12 autosomes, while 2*m* constituted the minute marker pair. Disregarding the size of the very minute *m*-pair, according to the approximate size of others the proportionality of the sex elements to autosomes was 5:12, taking each X_1 and the Y as equivalent to two X_2 in size. According to the number, it was 3 (X_1X_2Y):14 (12 autosomes plus the *m*-pair). These values could be used in calculating the expected number of aberrations between the sex chromosomes and autosomes.

3. Results

The chromosomes in different meiotic stages of the control specimens had practically no aberration because out of 274 spermatogonial metaphases, 1300 prophase I, 2600 metaphase I, 32 anaphase I and 2600 metaphase II assessed, only a gap type aberration was encountered. However, stickiness and clumping effects were not taken into account both in control and the treated specimens. The x-ray doses of 300 r, 400 r and 500 r in different meiotic stages induced the common aberrations like breaks (figures 1–3, 5, 6, 10, 11, 13, 15, 17), fragment of unknown origin (figures 11, 16), translocation/pseudochiasma (figures 1, 4, 7–9, 14), constriction (figures 9, 12) etc, while the anaphase I plates had specially the chromatid bridges (figure 18) seemed to be formed by the X_1 and/or the Y and some laggards. Like bridges in anaphase I, the pseudochiasma was specific to the prophase I and metaphase I.

An analysis of the x-ray induced aberration frequency data assessed at 13 different intervals for the treatment of 300 r (table 1) and 400 r (table 2) and at 500 r for 14 intervals (table 3) would reveal that because of the paucity of dividing cells, the chromosome aberrations were very likely not found in most of the intervals at spermatogonial metaphases of 300 r and 400 r (tables 1, 2) and to some extent in 500 r (table 3). Even then the aberration frequency in sex chromosomes was more than that of non-marker group in all doses. In 500 r the effect was present in all intervals between

Table 1. Frequency of aberrations in sex chromosomes and autosomes (figures in parentheses) induced by 300 r in different meiotic stages of *P. schlanbuschi*.

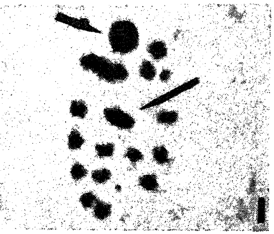
Fix. time	Sp. metaphase			Prophase I			Metaphase I			Anaphase I		Metaphase II		
	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Ana. bridge	No. of cells	Break	Other types
5 min	13	—	—	100	1	1	200	—	1	—	—	200	(1)	(1)
1 hr	5	—	—	100	1	2	200	2	1(1)	—	—	200	—	(1)
4 hr	40	1(2)	—	100	3	2	200	9(4)	5(1)	15	7	200	(1)	3(1)
8 hr	20	—	—	100	6	1(3)	200	11(4)	4(6)	5	4	200	(2)	1(4)
16 hr	62	—	3	100	8	10	200	5(2)	2(5)	3	—	200	4(2)	3(2)
24 hr	24	3(2)	—	100	3	3	200	3(2)	3(3)	—	—	200	1(3)	(3)
48 hr	27	—	—	100	1(1)	2(1)	200	2	10(1)	2	—	200	1	1
72 hr	12	—	—	100	1	5	200	5	6(2)	12	—	200	1	4
96 hr	28	—	5	100	2(1)	1	200	2	(1)	5	2	200	—	—
120 hr	15	—	—	100	8	7	200	10	1(2)	10	4	200	(2)	—
144 hr	23	—	—	100	4	—	200	4	1(2)	2	1	200	—	—
168 hr	9	1	—	100	3	3(1)	200	7	3	7	3	200	(2)	—
192 hr	29	—	—	100	1(1)	1	200	1	1(1)	5	3	200	—	1
Total	307	5(4)	8	1300	42(3)	38(5)	2600	61(12)	38(25)	66	24	2600	7(13)	13(12)

Table 2. Frequency of aberrations in sex chromosomes and autosomes (figures in parentheses) induced by 400 r in different meiotic stages of *P. schlanbuschi*.

	Sp. metaphase			Prophase I			Metaphase I			Anaphase I		Metaphase II		
Fix. time	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Ana. bridge	No. of cells	Break	Other types
5 min	5	—	1	100	(1)	—	200	—	2(2)	5	1	200	(1)	1(1)
1 hr	15	—	1	100	2	1	200	1(1)	2(3)	5	—	200	(2)	(1)
4 hr	9	—	—	100	2	7	200	9(3)	13(5)	4	1	200	—	(2)
8 hr	11	—	—	100	2(2)	6(7)	200	3(3)	12(5)	15	9	200	1	2(1)
16 hr	160	15(2)	5(8)	100	—	4(2)	200	8(4)	9(5)	2	1	200	—	1
24 hr	12	—	—	100	—	(2)	200	4(3)	12(5)	7	3	200	(3)	(2)
48 hr	9	—	—	100	—	—	200	5(2)	13(2)	8	7	200	(1)	—
72 hr	16	—	—	100	5	4	200	8	3(2)	6	4	200	(2)	—
96 hr	16	—	—	100	2	3(1)	200	15	2	4	—	200	—	—
120 hr	27	1	—	100	4(1)	7	200	6(2)	12(2)	4	3	200	1(1)	—
144 hr	—	—	—	100	3(2)	3	200	3	3	7	—	200	(1)	—
168 hr	10	—	—	100	2	3	200	7	6(2)	—	—	200	—	—
216 hr	6	—	—	100	8	—	200	5	1	3	1	200	(2)	—
Total	296	16(2)	7(8)	1300	30(6)	38(12)	2600	74(18)	90(33)	70	30	2600	2(13)	4(7)

Table 3. Frequency of aberrations in sex chromosomes and autosomes (figures in parentheses) induced by 500 r in different meiotic stages of *P. schlanbuschi.*

	Sp. metaphase			Prophase I			Metaphase I			Anaphase I		Metaphase II		
Fix. time	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Ana. bridge	No. of cells	Break	Other types
5 min	—	—	—	100	—	(2)	200	2	3(2)	—	—	200	—	—
1 hr	10	—	—	100	1	2(4)	200	2	2(2)	—	—	200	—	1
4 hr	28	6(2)	1(1)	100	—	11(6)	200	9(5)	13(9)	10	4	200	—	4
8 hr	93	12(1)	10(6)	100	(1)	3(3)	200	6(4)	15(9)	2	—	200	—	—
16 hr	60	13	9(6)	100	2	4(4)	200	5(3)	9(1)	—	—	200	—	—
24 hr	40	1(1)	1	100	1(1)	(1)	200	16(4)	20(6)	8	—	200	(1)	—
48 hr	13	—	1	100	(3)	1(3)	200	12(2)	19(2)	3	—	200	(2)	—
72 hr	10	—	—	100	6	—	200	7(2)	5(2)	5	2	200	(1)	(1)
96 hr	11	—	—	100	3(1)	2	200	5(4)	8(2)	—	—	200	—	—
120 hr	32	5	2(2)	100	1(2)	—	200	12(3)	—	3	1	200	—	—
168 hr	2	1	—	100	4(1)	—	200	19	2(2)	—	—	200	—	—
192 hr	—	—	—	100	—	—	200	1(1)	10(1)	3	1	200	(2)	—
216 hr	39	6	(3)	100	3(1)	1(2)	200	11(1)	3(3)	2	1	200	—	—
336 hr	22	—	—	100	—	—	200	2	—	—	—	200	(1)	—
Total	360	44(4)	24(18)	1400	21(10)	24(25)	2800	109(29)	109(41)	36	9	2800	(7)	5(1)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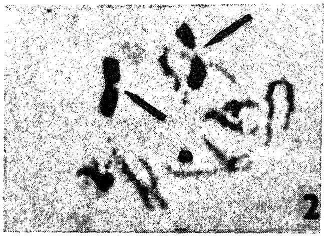
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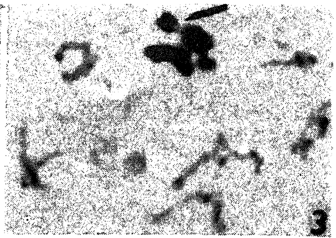
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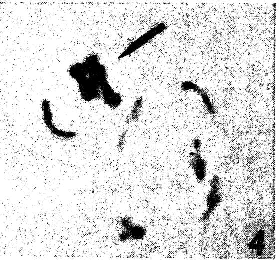
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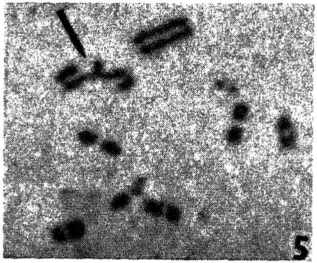
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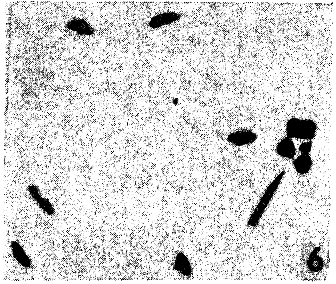
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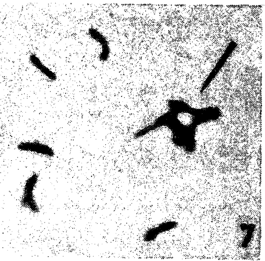
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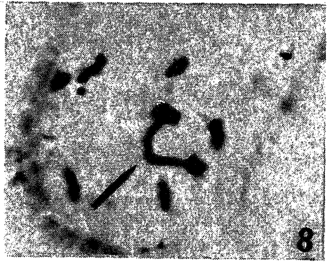
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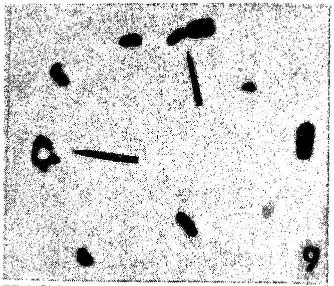
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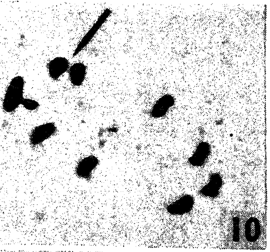
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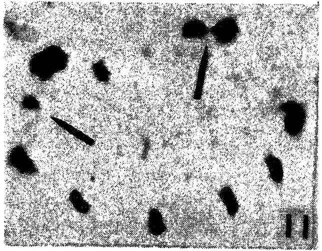
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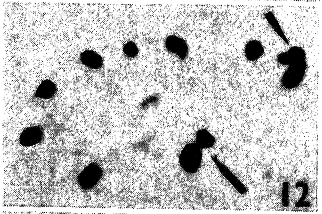
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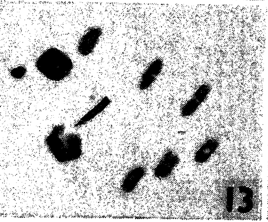
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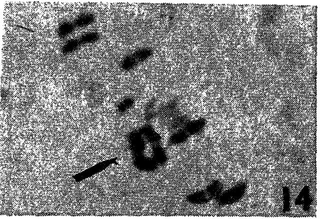
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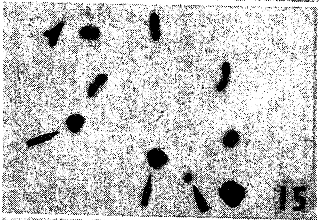
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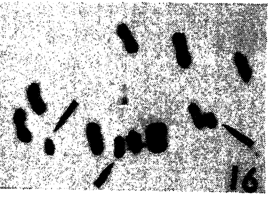
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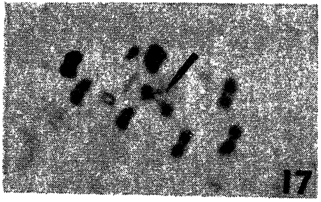
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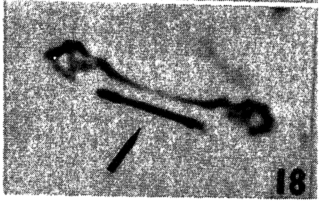
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4 hr and 24 hr with a peak at 16 hr, while it was rare and erratic at later intervals. The absence of fragment after 24 hr in all doses did not bear testimony to the holocentric nature of the chromosomes. Further, though the number of plates in anaphase I was also limited, the chromosomes appeared more vulnerable than those of the spermatogonial metaphases as revealed by the chromosome bridges formed mainly by the marker pair of sex chromosomes and sometimes laggards or other type of aberration. First two types of aberrations were also unexpected for the holocentric chromosomes (Manna 1984). Cells in prophase I, metaphase I and metaphase II were adequate in number and most of the intervals of fixation contained some form of aberrations (tables 1–3). When the aberration frequency at different intervals induced by 300 r, 400 r and 500 r was examined, a regular mode in rise and fall of the frequency could not be seen. The peak frequency was also found not at the same fixation interval in all these stages. It was 16 hr in prophase I, 8 hr in metaphase I and 16 hr in metaphase II for 300 r (table 1); 8 hr in prophase I and 4 hr in metaphase I and 24 hr in metaphase II for 400 r (table 2) and 4 hr in prophase I, 24 hr in metaphase I and indefinite in metaphase II for 500 r (table 3). In the pooled data of aberration frequency of all intervals, there was a disproportional increase in the higher doses except for the data of prophase I and metaphase II where the frequency was decreased in higher doses. Thus, for the doses 300 r, 400 r and 500 r respectively in the spermatogonial metaphases, it was 5·5% (17 out of 307), 11·1% (33 out of 296) and 25·0% (90 out of 360); in prophase I, 6·7% (88 out of 1300), 6·6% (86 out of 1300) and 5·7% (80 out of 1400); in metaphase I, 5·2% (136 out of 2600), 8·2% (31 out of 2600) and 10·2% (288 out of 2800); in anaphase I, 36·3% (24 out of 66), 42·3% (30 out of 70) and 25·0% (9 out of 36) and in metaphase II, 1·7% (45 out of 2600), 0·9% (25 out of 2600) and 0·5% (13 out of 2800). However, when the aberration data of all stages were combined, it was 4·5% (310 out of 6873) in 300 r, 5·6% (389 out of 6866) in 400 r and 6·4% (480 out of 7396) in 500 r, indicating an average of 1% increase for the rise of 100 r from 300 r to 500 r. Some erratic results obtained sometimes with regard to the dose dependent effect were possibly due to various factors as the analysis was made from the pooled data of various types of aberrations and of different intervals, some observational difficulty due to the physiological effect and so on.

Figures 1–18. Different stages of meiosis of X-irradiated *Physopelta schlanbuschi* showing various types of chromosome aberrations. **1.** Spermatogonial metaphase with a ring and a fragment formed by the marker sex chromosomes. **2.** Diplotene showing chromatid and isochromatid breaks in two marker sex chromosomes. **3.** Diplotene with 3 sex chromosomes associated one of which is broken in the mid-region. **4.** Diplotene showing a chiasma-like configuration formed by the X_1 and the Y, while the X_2 is attached to them. **5.** Diakinesis showing a chromatid break in the mid-region of a marker sex chromosome. **6.** Prometaphase I showing a break in the mid-region of a marker sex chromosome. **7.** Metaphase I showing an interstitial chiasma-like configuration formed by the X_1 and the Y. **8.** Showing the stretched out sex chromosome pair. **9.** Metaphase I showing a translocation between two autosomes and a constriction in a marker sex chromosome. **10.** Metaphase I showing a marker sex chromosome broken in the mid-region. **11.** Metaphase I with a break in the mid-region of a marker sex chromosome, while the X_2 is near another marker and a fragment of unknown origin. **12.** Metaphase I with a constriction in marker sex chromosome, while the X_2 is associated with another marker. **13.** Metaphase with a marker sex chromosome broken in the terminal region. **14.** Metaphase I with mid-region break in two associated marker pair. **15.** Metaphase I with dispersed broken parts of a marker sex and a fragment of unknown origin. **16** and **17.** Metaphase II each with some extra elements. **18.** Anaphase I showing two stout chromatid bridges.

Table 4. Observed and expected number of aberrations induced by 300 r, 400 r and 500 r doses in *P. schlanbuschi.*

Dose		Sp. metaphase		Prophase I		Metaphase I		Metaphase II		Total	
		Sex chromosome	Autosome	Sex chromosome	Autosome	Sex chromosome	Autosome	Sex chromosome	Autosome	Sex chromosome	Autosome
300 r	Obs.	13	4	80	8	99	37	20	25	212	74
	Exp.	5	12	26	62	40	96	13	32	84	202
400 r	Obs.	23	10	68	18	164	51	6	19	261	98
	Exp.	10	23	25	61	63	152	7	18	105	244
500 r	Obs.	68	22	45	35	218	70	5	8	336	135
	Exp.	27	63	24	56	85	203	4	9	172	299
Total	Obs.	104	36	193	61	481	158	31	52	809	307
	Exp.	42	98	75	179	188	451	24	59	361	745

With a view to finding out if there was any differential response of the sex chromosomes and autosomes, the aberration frequency in spermatogonial metaphase, prophase I, metaphase I and metaphase II induced by the 3 doses of x-rays was analysed (table 4) which revealed that the observed number of aberrations in the sex chromosomes, occurring mainly in the X_1 and the Y was relatively quite high than that of the expected number calculated on the proportionality of size (5 : 12) and the reverse was true for autosomal aberrations. It was found that in 300 r, 400 r and 500 r and in the total of all doses respectively the observed number of aberrations in the sex chromosomes was higher than the expected number by about 2·6 times, 2·3 times, 2·5 times and 2·5 times in spermatogonial metaphases; about 4 times, 2·7 times, 1·4 times and 2·5 times in prophase I; 2·3 times, 2·4 times, 2·5 times and 2·5 times in metaphase I, and 1·5 times, 1·1 times (lower), 1·2 times and 1·3 times in metaphase II. If the aberration data of the sex chromosomes in these 4 stages were combined, the observed number as compared to the expected number was higher by about 2·5 times in 300 r and 400 r and 1·9 times in 500 r and 2·2 times in the average of all doses, indicating thereby that the sex chromosomes were highly sensitive to the effect of the 3 doses of x-rays. On the other hand, the autosomes were found quite resistant when the observed and the expected numbers were compared except some erratic situation in metaphase II. Thus, in 300 r, 400 r, 500 r and the combined data of all doses respectively the observed number was lower by about 3 times, 2·3 times, 3 times and 2·7 times in the spermatogonial metaphase; about 1·7 times, 3·4 times, 1·6 times and 2·9 times lower in prophase I; 2·6 times, 3 times, 2·9 times and 2·8 times lower in metaphase I, while in metaphase II it was lower by 1·3 times, higher by 1·0 times and 1·1 times and lower by 1·1 times in the average. However, in the combined data of all stages the observed number of aberrations in autosomes was significantly lower than the expected number by 2·7 times in 300 r, 2·5 times in 400 r, 2·2 times in 500 r and 2·4 times in the pooled data of all doses indicating clearly that the aberrations were nonrandomly distributed. Further, an analysis of the breaking points in the X_1 and the Y also showed that the mid-region was more vulnerable than the two end regions if the said chromosomes were arbitrarily divided in 3 equal regions.

4. Discussion

In the present study, the aberration frequency in the germinal chromosomes of *P. schlanbuschi* showed on the whole that the effect was dependent on different doses of x-rays used in spite of some erratic results obtained in some stages. It was mentioned before that the frequency-increase was even directly proportional when the data of all stages were pooled, showing an increase of about 1 % aberration for an increase of 100 r as it was 4·5 % in 300 r, 5·6 % in 400 r and 6·4 % in 500 r. However, it was not found when the data of different stages were separately analysed, some reasons for which were stated before.

The present study did not favour the holocentric nature of chromosomes in *Physopelta* as the number of fragments neither increased nor could they survive in cells fixed at later intervals by which time the cell could have undergone a mitotic cycle. There was also a number of chromatid bridges and some laggards at anaphase I which was not expected for dividing holocentric chromosomes (Manna 1984, 1985). Further, the breaks in marker sex chromosomes of *Physopelta* were nonrandomly distributed etween and within chromosome complement as found in chromosomes of other pecies with localized centromere (Kihlman 1966; Manna 1975). Lastly the occurrence f the chiasma-like configuration between X_1 and the Y, found sometimes during rophase I and its persistence in the interstitial region in metaphase I was contrary to he characteristic regular behaviour of the holocentric chromosomes. The induction of hiasma-like configuration between the marker sex chromosomes found in prophase I vhich continued to the metaphase I has not been recorded so far in Heteroptera. It was uggested to be due to their close association during early prophase I and the x-ray had lelayed their separation otherwise the sex chromosome normally lie independently rom pachytene-diplotene stage (Manna and Dey 1981). It was also suspected by these vorkers (Manna and Dey 1981) that the genetical exchange between sex chromosomes night normally take place during very early prophase I when they remain associated ery closely. But uptill now no sex-linked genes in *Physopelta* have been discovered vhich could be used in verifying the proposition of genetical crossing-over of sexinked genes during very early prophase I.

That the sex chromosomes were more susceptible to x-irradiation than the utosomes was found in spermatocyte chromosome of grasshoppers (Manna and Aazumder 1962, 1967). Bick and Brown (1975) also showed that the sex chromosomes vere more radiosensitive than autosomes in cultured cells of a marsupial. Since the sex hromosomes are heterochromatic, it is tempting to suggest that they could be more ensitive to x-rays because of their heterochromatic nature.

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Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton

M SRIRAMULU* and K N MEHROTRA

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India

*Present address: Department of Entomology, A.P. Agricultural University, Hyderabad 500030, India

MS received 29 July 1985

Abstract. DNA content and effect of diflubenzuron was studied colorimetrically in developing pupae of *Corcyra cephalonica* Stainton. The DNA content in developing pupa followed a unique U-shaped curve. Diflubenzuron has no effect on DNA content in the pupae of *Corcyra cephalonica.*

Keywords. DNA; d1 diphenylamine; histolysis; histogenesis; diflubenzuron.

1. Introduction

The basic mechanism underlying growth and differentiation is protein synthesis, and is directly related to RNA which is in turn DNA dependent (Chen 1971). DNA content in insects has been studied extensively. In pupae of *Calliphora erythrocephala*, the nucleic acid content increased to about 25% during histogenesis. Final decrease was related to decomposition of DNA during lysis of fat body (Agrell 1952). Doubling of DNA in wing tissues and no change in fat bodies was observed during transition from diapause to imaginal development in pupae of *Hyalophora cecropia* silk moth (Linzen and Wyatt 1964). Howells and Birt (1964) reported reduced DNA content one day preceding pupation in *Lucilia cuprina* and there after a steady increase to a maximum just before emergence. Devi *et al* (1963) also observed increased DNA content in pupal stage of *Tribolium confusum*. But Choudhary and Lemonde (1966) reported no change in DNA content in *Tribolium confusum* pupal stage. About 25% reduction in DNA content was observed from early to late pupal stage in *Aedes aegypti* (Lang *et al* 1965) and in *Drosophila melanogaster* (Church and Robertson 1966). It is interesting to note that various species of insect pupae have their own pattern of DNA content during development. The present studies were therefore undertaken to know the pattern of DNA content in pupae of *Corcyra cephalonica* and the effect of diflubenzuron, a chitin synthesis inhibitor on DNA content.

2. Material and methods

Standard DNA (calf-thymus) was obtained from Sigma Chemical Company, St. Louis, Missouri, USA. Technical grade diflubenzuron was a gift from Dr A B Borkovec, Insect Chemosterilants Laboratory, US. Department of Agriculture, Beltsville, Maryland, USA. The pupae of *Corcyra cephalonica* Stainton (Pyralidae: Lepidoptera) were obtained from a laboratory colony maintained on broken *Sorghum bicolor* (L.) grains.

2.1 *Extraction of DNA*

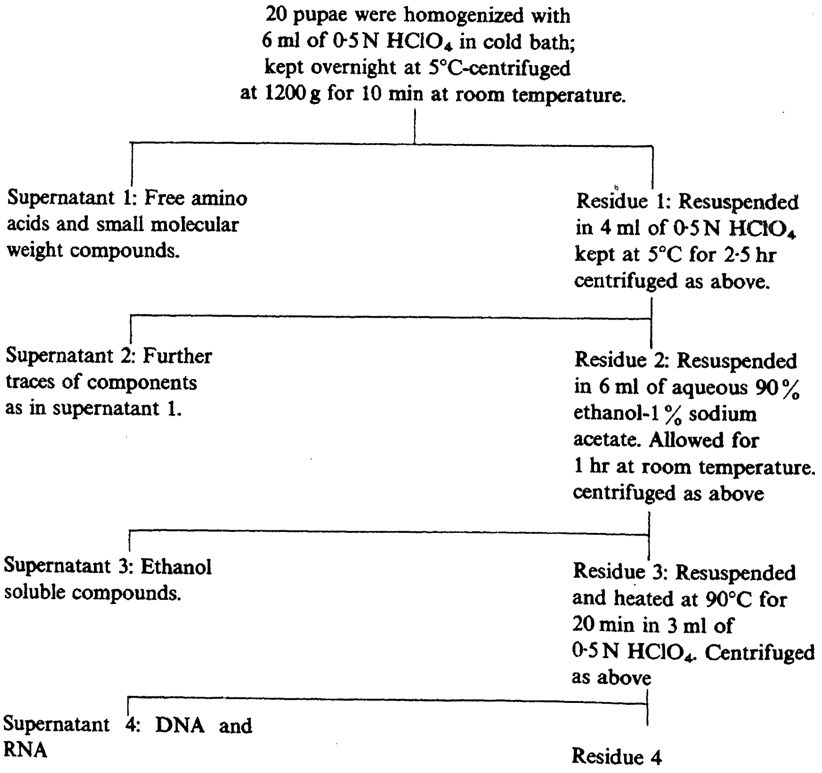
DNA was extracted by the method of Price (1969) which was based on method described by Schneider (1945) earlier, with a slight modification as shown in figure 1.

2.2 *Estimation of DNA*

DNA content was estimated colorimetrically using diphenylamine reagent (DPA reagent) as described by Dische (1930). DPA reagent was prepared by dissolving 1 g of diphenylamine in 100 ml of glacial acetic acid and 2·75 ml of concentrated sulphuric acid. One ml of extract was added to 2 ml of DPA reagent. The mixture was heated in a boiling water bath for 10 min. After 30 min, the intensity of blue colour was measured at 595 nm using ECIL spectrophotometer (Model G.S. 865). Calf-thymus DNA was used as standard.

2.3 *Recovery test*

To find out the efficiency of extraction method of DNA, a recovery test was conducted. The homogenized sample was fortified with a known amount of standard DNA and the



20 pupae were homogenized with 6 ml of 0·5 N $HClO_4$ in cold bath; kept overnight at 5°C-centrifuged at 1200 g for 10 min at room temperature.

Supernatant 1: Free amino acids and small molecular weight compounds.

Residue 1: Resuspended in 4 ml of 0·5 N $HClO_4$ kept at 5°C for 2·5 hr centrifuged as above.

Supernatant 2: Further traces of components as in supernatant 1.

Residue 2: Resuspended in 6 ml of aqueous 90% ethanol-1% sodium acetate. Allowed for 1 hr at room temperature. centrifuged as above

Supernatant 3: Ethanol soluble compounds.

Residue 3: Resuspended and heated at 90°C for 20 min in 3 ml of 0·5 N $HClO_4$. Centrifuged as above

Supernatant 4: DNA and RNA

Residue 4

Figure 1. Extraction of DNA from *C. cephalonica*.

DNA was extracted in both fortified and unfortified samples. Recovery was calculated based on difference in DNA of fortified and unfortified samples, representing the amount of DNA used for fortification (table 1). The recovery varied from 77·5–97·2% with a mean value of 82·4 + 6·8%.

2.4 *Diflubenzuron treatment*

To study the effect of diflubenzuron (a chitin synthesis inhibitor) on DNA content, the insecticide 0·1 μg per pupae (ED_{80}) was injected at 0 day of pupal age using dimethylformamid (DMFA) and methanol at 2:3 ratio as solvent. The material was injected to pupae with the help of a alcohol sterilized 27 gauge needle attached to 1 ml tuberculin syringe (Top syringe Manufacturing Company, Japan) using an electrically operated micro-applicator (Model M 1025, Instrumentation Specificities Company, USA). In both diflubenzuron treated and sham injected control pupae, DNA was extracted and estimated colorimetrically as described earlier.

3. Results

3.1 DNA *content during pupal development*

The data on DNA content in a normally developing pupa of *C. cephalonica* are presented in table 2 and figure 2. From the data it would be seen that the DNA content in the developing pupae followed a U-shaped curve. The DNA content decreased gradually from the time of pupation to 3rd day of pupal development and then increased. At physiological age of 0 day, the DNA content was 24·1 μg/pupa and gradually decreased to 23·8 μg at 1 day, 22·6 μg at 2 days and 21·8 μg at 3 days of pupal growth. From the 3rd day onwards DNA content increased to 22·4 μg at 4th day and 23·2 μg at 5th day of pupal development. The 0 and 1 day old pupae recorded significantly more DNA than 3 day old

Table 1. Per cent recovery of DNA

DNA content (μg)		
Fortified	Unfortified	Recovery (%)
100·2	65·7	86·3
114·0	82·0	80·0
118·2	87·5	76·7
125·3	86·4	97·2
113·4	82·3	77·7
109·8	78·8	77·5
154·0	121·4	81·5
	Mean	82·4
	SD	6·8

Homogenized sample was fortified with 40 μg of standard DNA and DNA was extracted in both fortified and unfortified samples as per the scheme (figure 1). The difference in DNA of fortified and unfortified samples represent 40 μg of DNA used for fortification.

Table 2. DNA content in the pupa of *C. cephalonica* during development

Pupal age (days)	DNA content	
	µg/pupa	µg/g body weight
0	24·1 ± 2·1	600·8 ± 58·9
1	23·8 ± 2·0	699·3 ± 59·2
2	22·6 ± 2·2	680·4 ± 80·5
3	21·8 ± 1·6	673·6 ± 48·0
4	22·4 ± 1·8	699·5 ± 59·1
5	23·2 ± 1·6	755·5 ± 57·7

Each value reported is the mean of 20 pupae with 10 replications each.

Statistically significant differences were observed between 0 and 3 days; 1 and 3 days in µg/pupa basis and 5th day and all other ages in µg/g body weight.

DNA was extracted by the method of Price (1969) as described in figure 1. It was estimated colorimetrically after reaction with diphenylamine.

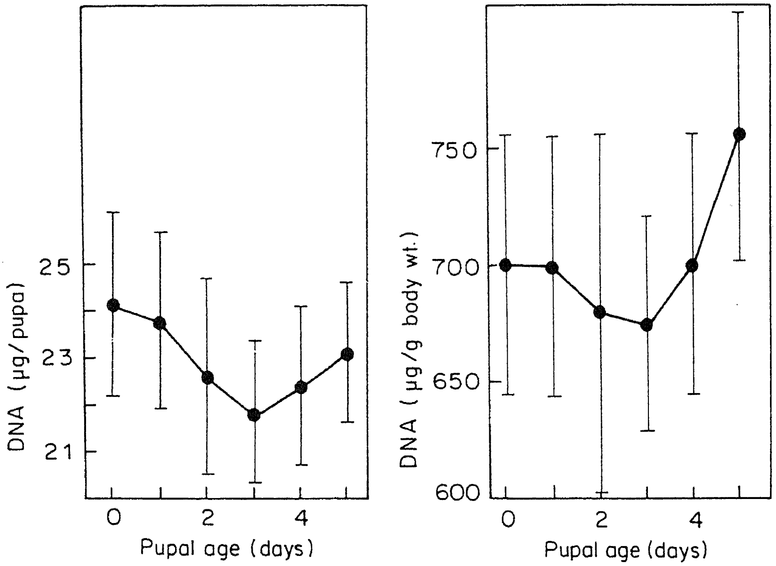


Figure 2. DNA content in the pupae of *C. cephalonica* during development.

pupa. While the difference in DNA content at other physiological ages was not statistically significant.

Because of loss in weight during pupal development, the DNA content per g body weight was also calculated. From the data presented in table 2 and figure 2, it would be seen that it also followed the U-shaped curve. The DNA content decreased from

699·8 μg/g body weight at 0 day to 673·6 μg/g body weight on 3rd day of pupal development. From 3rd day onwards DNA content increased to 755·5 μg/g body weight on 5th day of pupal development. The 5th day pupae recorded significantly more DNA than all other physiological age pupae while the difference in DNA content at other physiological ages was not significant.

On g body weight, 5th day pupa recorded more DNA content than 0 day pupa and on pupal basis 0 day pupa recorded more DNA content than 5th day pupa. This difference in DNA pattern was apparently due to loss in weight during pupal development.

3.2 *Effect of diflubenzuron on DNA content during pupal development*

The data on DNA content in the solvent-injected control and diflubenzuron treated pupae are presented in table 3 and figure 3. In both control and diflubenzuron treated pupae the pattern of DNA content confirmed the earlier results (table 2 and figure 2). The pattern of DNA content in control and diflubenzuron treated pupae were the same and followed a U-shaped curve. A gradual reduction in DNA content was observed from the time of pupation to 3 days of pupal development and then increased. At physiological age of 0 day the DNA content was 23·1 μg/pupa in control and 22·8 μg/pupa in diflubenzuron treated pupa. The DNA content gradually decreased to 19·2 μg/pupa in control and 19·5 μg/pupa in diflubenzuron treated pupa. The DNA content gradually decreased to 19·2 μg/pupa in control and 19·5 μg/pupa in diflubenzuron treated pupa at 3rd day of pupal development. From 3rd day the DNA content increased to 21·1 μg/pupa in control and 21·6 μg/pupa in diflubenzuron treated pupa at 5th day of pupal development.

The DNA content expressed on per g body weight basis also followed a U-shaped curve in both control and diflubenzuron treated pupae. The difference in DNA content in control and diflubenzuron treated pupae at various developmental stages of pupa was not statistically significant.

Table 3. Effect of diflubenzuron on DNA content of *C. cephalonica* pupa.

Pupal age (days)	DNA content			
	μg/pupa		μg/g body weight	
	Control	Treated	Control	Treated
0	23·1 ± 1·2	22·8 ± 0·9	675·8 ± 52·5	666·2 ± 31·8
1·0	20·4 ± 1·3	21·2 ± 0·7	615·8 ± 42·5	625·7 ± 23·3
2·0	19·8 ± 0·9	19·8 ± 0·6	615·2 ± 33·5	614·1 ± 23·2
2·5	20·4 ± 0·8	19·9 ± 0·6	629·2 ± 26·1	624·8 ± 22·9
3·0	19·2 ± 0·8	19·5 ± 1·0	602·2 ± 26·3	614·4 ± 33·7
3·5	20·3 ± 0·9	20·2 ± 0·7	647·5 ± 21·9	613·8 ± 22·3
4·0	20·1 ± 1·5	20·3 ± 0·8	635·6 ± 48·9	640·7 ± 31·3
4·5	20·9 ± 0·6	20·4 ± 1·1	648·3 ± 20·9	669·6 ± 25·7
5·0	21·1 ± 0·8	21·6 ± 0·7	682·9 ± 29·2	690·6 ± 24·2

Each value reported is the mean of 10 pupae with 6 replicates each.

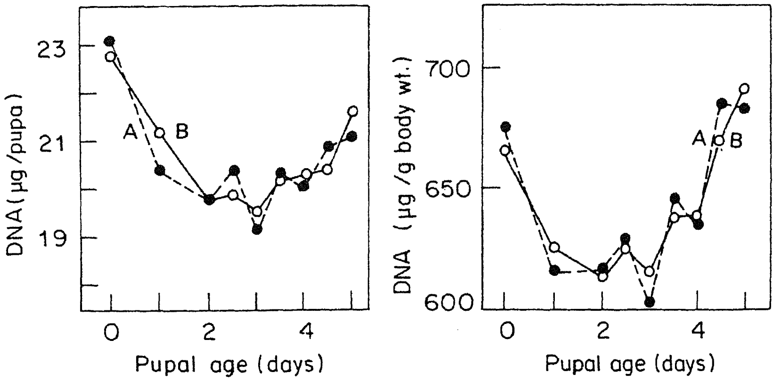


Figure 3. Effect of diflubenzuron on DNA content in *C. cephalonica* pupa. (A) control, (B) treated.

4. Discussion

4.1 DNA content during pupal development

DNA content in insects has been studied extensively (Agrell 1952; Devi *et al* 1963; Linzen and Wyatt 1964; Howells and Birt, 1964; Lang *et al* 1965; Choudhary and Lemonde 1966; Church and Robertson 1966). It is interesting to note that various species of insect pupae have their own pattern of DNA content during development. The different patterns observed are (i) increase in DNA content in early stage of pupal development followed by a decrease at the final stage as has been reported in *Calliphora erythrocephola* (Agrell 1952), (ii) no change in DNA content throughout the pupal period as has been reported in *Tribolium confusum* (Choudhary and Lemonde 1966), (iii) reduction in DNA content during pupal development as has been reported in *Aedes aegypti* (Lang *et al* 1965; Church and Robertson 1966). In the present study *C. cephalonica* was shown to have a unique pattern and that DNA content followed a U-shaped curve during development. These contradictory reports may perhaps be due to differences in physiological development in different species of insects.

The decrease in DNA content upto 3 days most likely corresponded with the period of histolysis of various organs while the subsequent rise in DNA contents were perhaps due to histogenesis and differentiation of various tissues for the future adult. Indeed, the results reported confirm the findings of Howells and Birt (1964) who observed that in *Lucilia cuprina* the total DNA declined rapidly one day preceding pupation and thereafter increased steadily to a maximum just before adult emergence. Howells and Birt (1964) suggested that this pattern was related to disintegration of larval tissue (histolysis) at early pupal stage followed by proliferation of imaginal cells (histogenesis) during later stage of development. It is also likely that decrease in DNA in early pupal stage might have been due to high DNAase concentration. In fact, Muhammed *et al* (1967) have reported a high titre of DNAase activity in late 3rd instar larvae of *D. melanogaster*, parallel to the onset of break down of larval tissues and a rapid drop to a low level during the first two days of pupal life, when adult differentiation is under way. Thus the decrease in DNA in early stages and increase in later stages can be reasonably explained due to histolysis in early part of pupal life and histogenesis in later part.

4.2 *Effect of diflubenzuron on DNA content*

The results on DNA content of diflubenzuron treated pupae and solvent injected control were in no way different from each other and followed a unique U-shaped curve. Similar findings were also reported in the early stage of *Stomoxys calcitrans* pupae by De Loach *et al* (1981). They however, did not study the effect of diflubenzuron on DNA content at the later stage of pupal development. The results reported suggest that diflubenzuron treatment did not materially affect the DNA content in the pupae of *C. cephalonica.*

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Hematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters)

M A HANIFFA, A G MURUGESAN and M PORCHELVI

Post Graduate and Research Department of Zoology, St. Xavier's College, Palayamkottai 627 002, India

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Abstract. Freshwater fish *Heteropneustes fossilis* and *Sarotherodon mossambicus* were exposed to distillery and paper mill effluents with a view to check the hematological changes as a function of feeding/starvation and aeration/non-aeration. Increase in concentration always produced elevated values in RBCs irrespective of feeding and/or aeration. This elevation was statistically significant ($P < 0.05$) in most of the test individuals. Fish exposed to effluents showed a dose dependent increase in WBCs ($P < 0.05$). Maximum number of RBCs and WBCs was always noticed in the non-aerated condition. Statistical analyses confirmed that the change in number of blood cells as a function of feeding/starvation and aeration/non-aeration was not significant ($P > 0.05$).

Keywords. Distillery and paper mill effluents; fish; hematology.

1. Introduction

Industrial effluents and sewage are the major pollutants contaminating our aquatic systems. Distillery, pulp and paper mill effluents have been of major concern in water pollution abatement programmes primarily because fish communities are affected by suspended solids (Sprague and Mcleese 1968; Verma and Dalela 1975), oxygen consuming materials (Blinski and Jonas 1973; Johnson 1977) and taste and odor creating substances (Shymway and Palensky 1973; Thomas 1973). Numerous physiological, biochemical and histochemical changes in the blood and tissues of fish exposed to a variety of pollutants have been reported (Warner 1967; Sprague 1971; Katz *et al* 1979). But only few authors gave due importance to study the quantitative measurement of specified physiological and biochemical changes in the blood and tissues of fish exposed to industrial effluents (Mcleay 1973; Larsson *et al* 1980; Murugesan and Haniffa 1985). In this regard little is known about hematological changes of fish exposed to distillery effluent which contains magnesium (1506 mg/l), phosphate (520 mg/l), sulphate (3600 mg/l), chloride (5600 mg/l), sodium (1700 mg/l), calcium (561 mg/l) and potassium (885 mg/l). Blood constituents are also affected by different factors such as starvation (Sharma *et al* 1978) and aeration (Hoar 1976). Hence in the present investigation 4 different conditions, viz aerated fed, aerated starved, non-aerated fed and non-aerated starved have been chosen and the hematological effects have been determined in two freshwater fishes *Heteropneustes fossilis* and *Sarotherodon mossambicus* exposed to distillery and paper mill effluents.

2. Materials and methods

The test fish *H. fossilis* (15 ± 2 g live weight) and *S. mossambicus* (12 ± 2 g live weight) were collected from the local ponds and acclimated to laboratory conditions and fed on beef slices (Haniffa and Sundaravadhanam 1983). Decided sublethal concentrations of distillery effluent*, viz 0·05%, 0·5% and 1% concentrations were prepared using dechlorinated tap water. Sixteen *H. fossilis* of the size 15 ± 2 g live weight were recruited from the stock and divided into 4 groups each consisting of 4 individuals. The first group was fed on beef slices *ad libitum* twice a day and water was aerated daily. The remaining 3 groups were exposed to aerated starved, non-aerated fed and non-aerated starved conditions, respectively. Every day fresh media were supplied to the test individuals and the fish were maintained in these media for a period of one month at 28 ± 2°C.

At the end of the exposure period, the fish were sacrificed with a blow on the head and blood was collected from the caudal vein in asceptic condition by means of a standard micropipette. The total RBCs and WBCs were counted using Haemocytometer and Neubauer counting chamber. The same procedure was followed for *S. mossambicus* exposed to chosen sublethal concentrations of distillery and paper mill** effluents.

3. Results and discussion

Table 1 reports the total number of RBCs of *H. fossilis* exposed to different concentrations of distillery effluent. When compared with the starved individuals, *H. fossilis* fed *ad libitum* always showed an increase in RBCs ($P > 0{\cdot}05$). This elevation was more pronounced when the individuals were reared in non-aerated condition. Increase in concentration always caused an increase in RBCs irrespective of aeration and/or starvation. This elevation was statistically significant in many cases. In the non-aerated condition both in fed and starved groups there was a 3-fold increase in RBCs from the lowest ($0{\cdot}9 \times 10^6/\mathrm{mm}^3$) to the highest concentration ($2{\cdot}3$–$2{\cdot}6 \times 10^6/\mathrm{mm}^3$); the same was only twofold in the aerated condition. *H. fossilis* reared in aerated fed and starved conditions showed an increase in RBCs from 1·04–2·03 and 0·79–1·55 × $10^6/\mathrm{mm}^3$ respectively. *S. mossambicus* reared in aerated fed condition showed an increase in RBCs from 0·89–1·27 × $10^6/\mathrm{mm}^3$ as a function of concentration. These elevated values ranged from 0·95–1·30, 1·06–1·44 and 1·04–1·62 × $10^6/\mathrm{mm}^3$ for the fish reared in the other 3 conditions (table 1).

H. fossilis exposed to paper mill effluent showed an increase in RBCs as a function of concentration. This increase was statistically significant in the aerated fed, aerated starved and non-aerated starved individuals. Here also the maximum number ($3{\cdot}84 \times 10^6/\mathrm{mm}^3$) was noticed for the non-aerated fed individuals. When compared with the starved *H. fossilis*, fish fed *ad libitum* showed more RBCs both in aerated and in non-aerated conditions (table 2). Increase in concentration of paper mill effluent produced elevated values in RBCs from 0·84–1·72 × $10^6/\mathrm{mm}^3$ in the aerated fed *S. mossambicus*; the same was from 1·73–2·05, 1·5–2·47 and 1·28–2·42 × $10^6/\mathrm{mm}^3$ for the other groups respectively. In the control fish, feeding caused an increase in RBCs in aerated and in non-aerated conditions in *S. mossambicus* but a decrease in the non-aerated *H. fossilis* (table 2).

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*Courtesy Trichy Chemicals and Distilleries Ltd., Tiruchirapalli.

**Courtesy Eagle Paper Mills, Poraiyur, Tirunelveli District.

Table 1. Effect of distillery effluent on number of RBCs of *H. fossilis* and *S. mossambicus*. All values are reported as $10^6/mm^3$.

Condition	Effluent concentration 0%	0·05%	0·5%	1%	Correlation coefficient (P)
H. fossilis					
Aerated fed	0·57 ± 0·03	1·04 ± 0·07	1·32 ± 0·23	2·03 ± 0·03	$<0{\cdot}05$
Aerated starved	0·53 ± 0·02	0·79 ± 0·07	1·36 ± 0·01	1·55 ± 0·05	$<0{\cdot}05$
	Feeding versus starvation				$P>0{\cdot}05$
Non-aerated fed	0·63 ± 0·05	0·95 ± 0·10	1·90 ± 0·30	2·65 ± 0·20	$>0{\cdot}05$
Non-aerated starved	0·71 ± 0·03	0·84 ± 0·01	1·42 ± 0·20	2·31 ± 0·01	$<0{\cdot}05$
	Aeration versus non-aeration				$P>0{\cdot}05$
		$P<0{\cdot}05^{*}$	$P<0{\cdot}01^{a}$ $P<0{\cdot}05^{b}$	$P<0{\cdot}01^{a}$ $P<0{\cdot}05^{b}$ $P>0{\cdot}05^{b}$	
	0%	0·025%	0·05%	0·1%	
S. mossambicus					
Aerated fed	0·71 ± 0·09	0·89 ± 0·10	0·97 ± 0·02	1·27 ± 0·20	$<0{\cdot}02$
Aerated starved	0·69 ± 0·02	0·95 ± 0·10	1·08 ± 0·03	1·30 ± 0·18	$>0{\cdot}05$
	Feeding versus starvation				$P>0{\cdot}05$
Non-aerated fed	1·02 ± 0·09	1·06 ± 0·04	1·23 ± 0·05	1·44 ± 0·10	$<0{\cdot}001$
Non-aerated starved	1·01 ± 0·20	1·04 ± 0·07	1·26 ± 0·01	1·62 ± 0·20	$<0{\cdot}001$
	Aeration versus non-aeration				
		$P>0{\cdot}05^{*}$	$P<0{\cdot}05^{a}$ $P>0{\cdot}05^{b}$	$P<0{\cdot}05^{a}$ $P<0{\cdot}05^{b}$ $P>0{\cdot}05^{c}$	$P>0{\cdot}05$

Student's '*t*' test (for effect of concentration) compared with [a]Control; [b]0·05% (0·025% for *S. mossambicus*); [c]0·5% (0·05% for *S. mossambicus*).
Values are mean ± SD from 3 individual observations.

The exposure of *H. fossilis* to the distillery effluent produced significantly enhanced level of WBCs (table 3). This dose dependent increase was always high for the test individuals reared in non-aerated condition. *H. fossilis* kept under aerated fed condition showed an increase in WBCs from 0·20–0·36 × $10^6/mm^3$ as a function of effluent concentration. These elevated values ranged from 0·16–0·34, 0·23–0·52 and 0·24–0·40 × $10^6/mm^3$ for the individuals of other groups respectively. *S. mossambicus* too showed a similar trend for the change in number of WBCs as a function of concentration. Except in the non-aerated starved condition, *S. mossambicus* reared in the other 3 conditions showed a significant dose dependent increase in WBCs. This increase was about 5–6 times for the test fish reared in aerated condition and was about 3 times for those reared in non-aerated conditions. Maximum WBCs were also noticed for the test fish reared under non-aerated fed condition.

Except in the aerated fed condition, the exposure of *H. fossilis* to the paper mill effluent produced significantly enhanced level of WBCs. Test fish reared in the maximum concentration of the effluent (1%) showed a 3-fold increase in WBCs irrespective of aeration or feeding. Feeding always caused increased values for WBCs in

Table 2. Effect of paper mill effluent on number of RBCs of *H. fossilis* and *S. mossambicus*. All values are reported as $10^6/\text{mm}^3$.

Condition	Effluent concentration 0%	0·05%	0·5%	1%	Correlation coefficient (*P*)
H. fossilis					
Aerated fed	0·57 ± 0·03	0·79 ± 0·06	1·58 ± 0·03	2·30 ± 0·02	< 0·05
Aerated starved	0·53 ± 0·02	0·76 ± 0·20	1·26 ± 0·07	1·74 ± 0·01	< 0·02
	Feeding versus starvation				
					P > 0·05
Non-aerated fed	0·53 ± 0·05	1·21 ± 0·01	2·32 ± 0·04	3·84 ± 0·01	> 0·05
Non-aerated starved	0·71 ± 0·03	1·08 ± 0·01	1·82 ± 0·03	2·96 ± 0·01	< 0·001
	Aeration versus non-aeration				
		P < 0·05[a]	*P* < 0·05[a] *P* < 0·05[b]	*P* < 0·05[a] *P* < 0·05[b] *P* > 0·05[c]	*P* > 0·05
S. mossambicus					
Aerated fed	0·71 ± 0·30	0·84 ± 0·20	1·37 ± 0	1·72 ± 0·01	< 0·02
Aerated starved	0·69 ± 0·02	0·73 ± 0·03	1·23 ± 0·06	2·05 ± 0·04	> 0·001
	Feeding versus starvation				
					P > 0·05
Non-aerated fed	1·02 ± 0·09	1·50 ± 0·02	1·80 ± 0·20	2·47 ± 0·02	> 0·05
Non-aerated starved	1·01 ± 0·20	1·28 ± 0·06	1·62 ± 0·20	2·42 ± 0·01	< 0·02
	Aeration versus non-aeration				
		P > 0·05[a]	*P* < 0·05[a] *P* > 0·05[b]	*P* < 0·05[a] *P* < 0·05[b] *P* > 0·05[c]	*P* > 0·05

Student's '*t*' test (for effect of concentration) compared with [a]Control; [b]0·05% (0·025% for *S. mossambicus*); [c]0·5% (0·05% for *S. mossambicus*).
Values are mean ± SD from 3 individual observations.

all the concentrations. But these elevated values however were not statistically significant, when all the 4 conditions were considered (table 4). *S. mossambicus* exposed to paper mill effluent also showed a dose dependent increase in the number of WBCs in all the 4 conditions. The increase was statistically significant for those fed *ad libitum* irrespective of aeration. The elevation was about 3–4 times greater for the test individuals kept in aerated condition and was 1·5–2 times greater for those kept under non-aerated condition. When compared with the aerated starved test fish reared in tap water, the aerated fed *H. fossilis* showed an increase in WBCs; the same trend was also noticed in the non-aerated condition (table 4).

The exposure of *H. fossilis* and *S. mossambicus* to the distillery and paper mill effluents produced significantly enhanced levels of erythrocytes and leucocytes. According to Haniffa and Isai Arasu (1984) *Mystus armatus* exposed to paper mill effluent showed increase in RBCs ($1·4–2·4 \times 10^6/\text{mm}^3$) and WBCs ($1·7–2·9 \times 10^4/\text{mm}^3$). Haniffa *et al* (1984) reported 4–5 times increase in RBCs of *H. fossilis* and *S. mossambicus* due to the exposure of textile mill effluent. Larsson *et al* (1980) reported a twofold increase in erythroblast of the flounders, *Platichthys flesus* exposed to

Table 3. Effect of distillery effluent on number of WBCs of *H. fossilis* and *S. mossambicus*. All values are reported as $10^6/\text{mm}^3$.

Condition	0%	0·05%	0·5%	1%	Correlation coefficient (*P*)
		Effluent concentration			
H. fossilis					
Aerated fed	0·13±0·01	0·20±0·05	0·33±0·02	0·36±0·03	>0·05
Aerated starved	0·11±0·02	0·16±0·02	0·29±0·10	0·34±0·05	<0·05
		Feeding versus starvation			
					P>0·05
Non-aerated fed	0·09±0·01	0·23±0·10	0·34±0·01	0·52±0·02	>0·05
Non-aerated starved	0·06±0·01	0·24±0·02	0·30±0·04	0·40±0·03	>0·05
		Aeration versus non-aeration			
					P>0·05
		P>0·05[a]	*P*<0·05[a]	*P*<0·001[a]	
			P>0·05[b]	*P*<0·001[b]	
				P>0·05[c]	
	0%	0·025%	0·05%	0·1%	
S. mossambicus					
Aerated fed	0·06±0·01	0·09±0·01	0·19±0·02	0·47±0·01	<0·02
Aerated starved	0·06±0	0·08±0·01	0·21±0·06	0·46±0·03	>0·05
		Feeding versus starvation			
					P<0·05
Non-aerated fed	0·09±0·04	0·20±0·01	0·47±0·01	0·63±0·02	<0·05
Non-aerated starved	0·09±0·03	0·16±0	0·48±0·07	0·56±0·01	>0·05
		Aeration versus non-aeration			
					P>0·05
		P>0·05[a]	*P*<0·05[a]	*P*<0·001[a]	
			P>0·05[b]	*P*<0·001[b]	
				P>0·05[c]	

Student's 't' test (for effect of concentration) compared with [a]Control; [b]0·05% (0·025% for *S. mossambicus*); [c]0·5% (0·05% for *S. mossambicus*).
Values are mean ± SD from 3 individual observations.

titanium dioxide effluent. The enhanced values reported by Pandey *et al* (1979) for the obligatory air-breathing fish *Channa punctatus* exposed to endrin ranged from 2·6–3·6 $\times 10^6/\text{mm}^3$. Mcleay (1973) reported that the increase in immature erythrocytes of juvenile coho salmon *Oncorhyncus kisutch* exposed to kraft pulp mill effluent could reflect a stimulation of erythropoiesis by elevated demands for oxygen or carbon dioxide transport in effluent exposed fish as a result of increased physical or metabolic activity or by destruction of gill membranes causing faulty gaseous exchange. Erythropoiesis could also be stimulated by an increased fragility or rate of destruction of circulating erythrocytes. In the present investigation fish exposed to paper mill effluent produced 3–8 times increase in WBCs (table 4). Even though aeration and feeding altered the number of blood cells, the student's '*t*' test analysis confirmed that the difference in number of cells due to feeding/starvation and aeration/non-aeration was not statistically significant ($P > 0·05$).

Table 4. Effect of paper mill effluent on number of WBCs of *H. fossilis* and *S. mossambicus*. All values are reported as $10^6/\text{mm}^3$.

Condition	Effluent concentration 0%	0·05%	0·5%	1%	Correlation coefficient (P)
H. fossilis					
Aerated fed	0·13 ± 0·01	0·13 ± 0·02	0·36 ± 0·01	0·39 ± 0·06	>0·05
Aerated starved	0·11 ± 0·02	0·12 ± 0·02	0·26 ± 0·06	0·30 ± 0·05	<0·05
	Feeding versus starvation				$P>0·05$
Non-aerated fed	0·09 ± 0·01	0·16 ± 0·06	0·31 ± 0·01	0·49 ± 0·01	<0·001
Non-aerated starved	0·06 ± 0·01	0·14 ± 0·03	0·29 ± 0·01	0·47 ± 0·01	<0·001
	Aeration versus non-aeration				$P>0·05$
		$P>0·05^{a}$	$P<0·05^{a}$	$P<0·01^{a}$	
			$P>0·05^{b}$	$P<0·05^{b}$	
				$P>0·05^{c}$	
S. mossambicus					
Aerated fed	0·06 ± 0·01	0·11 ± 0·01	0·23 ± 0	0·36 ± 0·03	<0·001
Aerated starved	0·06 ± 0	0·07 ± 0·03	0·10 ± 0·02	0·29 ± 0·03	>0·05
	Feeding versus starvation				$P>0·05$
Non-aerated fed	0·09 ± 0·04	0·43 ± 0·02	0·49 ± 0·03	0·71 ± 0·01	<0·001
Non-aerated starved	0·09 ± 0·06	0·42 ± 0·02	0·49 ± 0	0·62 ± 0	>0·05
	Aeration versus non-aeration				$P<0·05$
		$P>0·05^{a}$	$P>0·05^{a}$	$P<0·05^{a}$	
			$P>0·05^{b}$	$P>0·05^{b}$	
				$P>0·05^{c}$	

Student's '*t*' test (for effect of concentration) compared with ^{a}Control; ^{b}0·05% (0·025% for *S. mossambicus*); ^{c}0·5% (0·05% for *S. mossambicus*).

Values are mean ± SD from 3 individual observations.

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Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera)

K VENKATARAMAN*, M KESARY** and S KRISHNASWAMY

Department of Environmental Biology, School of Biological Sciences, and *School of Energy Environment and Natural Resources, Madurai Kamaraj University, Madurai 625 021, India
**Christian College, Martandam, Kanyakumari District 629 165, India

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Abstract. *Daphnia similis* Claus is a tropicopolitan and dominant species of temporary ponds of South India. Laboratory cultures were made to study the longevity, preadult instars, egg production and growth using different concentrations of rice bran, an agro-industrial waste which is available readily and cheaply. Rice bran dissolved in pond water resulted not only in higher fecundity but also in an additional increase in size and as such rice bran may form an ideal nutrient medium for mass production of daphnids. Egg production is 6 times higher and growth is two fold greater in cultures with rice bran in pond water than in tap water with rice bran. From the present study it is hoped that, the use of rice bran in mass culture of *Daphnia similis* will be profitable.

Keywords. *Daphnia similis;* longevity; egg production and body size; rice bran as food; laboratory conditons.

1. Introduction

In the present day application of biological sciences, *Daphnia* culture forms an integral part of the fishery development. The mass cultivation of *Daphnia* attracted the attention of many aquaculturists (Banta 1921; Bogatova 1951; Bogatova and Askerov 1958; Ivleva 1973; Murphy 1970; Harvey 1972; Bhanot and Vass 1976). Recently a successful cultivation of *D. magna* on rice bran was achieved by De Pauw *et al* (1981). Various types of feed are used in the mass culture of *Daphnia*. Cow-dung, poultry manure, green algae etc., which are used as feed in *Daphnia* culture are having high manure value in agriculture. The cow-dung is also used as a supplementary item for fire-wood in India. Moreover, cow-dung is in heavy demand for gobar gas plants. On the other hand the rice bran is an agro-industrial waste which is easily available in large quantities. It can be applied directly and is available at low price and can be stored for a long time. Above all rice bran forms an ideal feed for *Daphnia* enabling better growth and higher reproduction (De Pauw *et al* 1981). In the present experiment it is therefore aimed to study the effect of different concentration of rice bran, both in pond water and tap water on the growth and reproduction of a commonly occurring tropicopolitan species *Daphnia similis* Claus.

2. Material and methods

Ovigerous females of *D. similis* were collected from a seasonal pond near the University campus (Lat.: 9° 53′ N; Long.: 78° 8′ E) and were acclimated to the laboratory temperature (28–30°C). Just born neonates were separated from the mothers and were used for further studies. Table 1 shows the different culture media and the number of animals reared. The method used by Venkataraman (1981, 1983) was followed to study the life history. Values obtained at different experimental conditions were tabulated to find out the differences in growth and egg production statistically (Analysis of variance; Zar 1974), size at birth, average life span, number of instars, instar at initial reproduction (primiparous instar) and total number of eggs produced.

3. Results

The survivorship curve of *Daphnia* cultured at different experimental conditons are shown in figure 1. Average life span is observed to be higher in pond water with 3 different concentrations of rice bran than in other culture media. In the present study the number of preadult instar vary between 3–4 and the adult instar vary between

Table 1. Laboratory culture of *D. similis* under different experimental conditions.

Medium	Number of animals
Pond water	10
Pond water + 250 mg/l rice bran	10
Pond water + 500 mg/l rice bran	10
Pond water + 1000 mg/l rice bran	10
Tap water	10
Tap water + 250 mg/l rice bran	10
Tap water + 500 mg/l rice bran	10
Tap water + 1000 mg/l rice bran	10

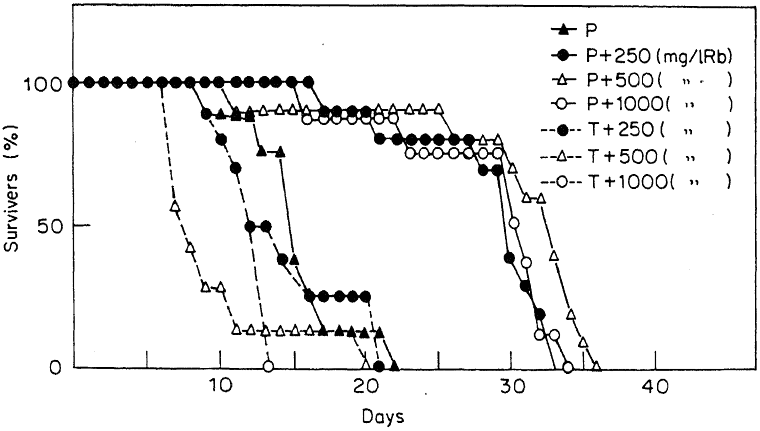

Figure 1. Survivorship curve of *D. similis* cultured under different experimental conditions. P, Pond water; T, tap water; Rb, rice bran.

8–22. Mean duration of first adult instar during which the females are primiparous is distinctly longer than the longest preadult instar.

Egg production is based on the mean number of young animals released from the brood pouch during each instar (figure 2). Maximum number of eggs have been obtained only during the earlier phase of life which gradually declined during the later stages. *D. similis* cultured in pond water with rice bran has a higher rate of reproduction ($a = 7{\cdot}98$ in 250 mg/l pond water + rice bran; $a = 30{\cdot}50$ in 500 mg/l pond water + rice bran; $a = 19{\cdot}65$ in 1000 mg/l pond water + rice bran) than in tap water with rice bran ($a = 0{\cdot}272$ in 250 mg/l; $a = 0{\cdot}70$ in 500 mg/l and $a = 5{\cdot}701$ mg/l) (figure 3). Table 2 shows the number of eggs produced in a life span of *D. similis* under different

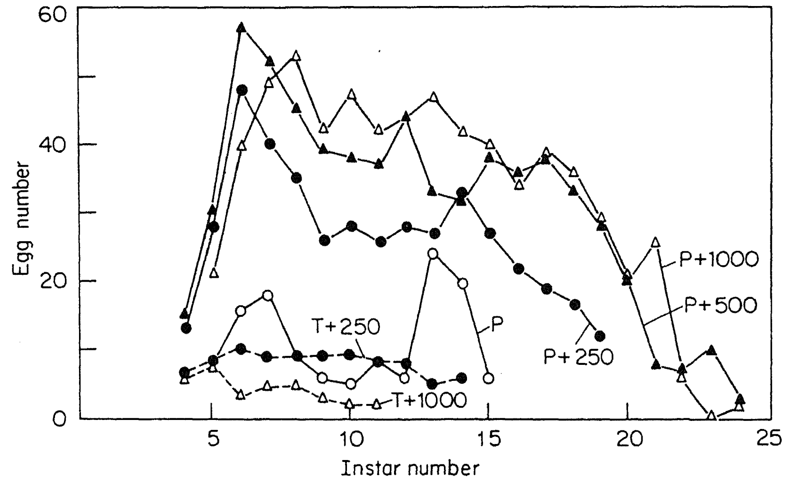

Figure 2. Comparison of egg number in relation to instar number of *D. similis* under different experimental conditions.

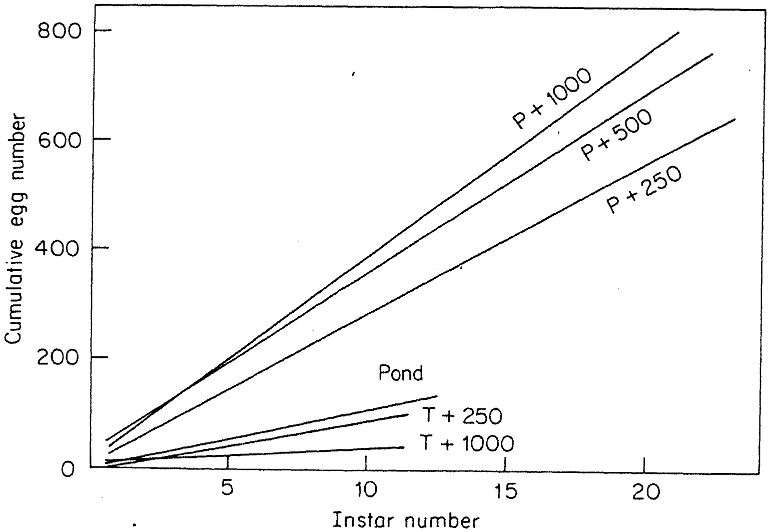

Figure 3. Regression analysis shows the cumulative egg number in relation to instar number of *D. similis* cultured in different concentrations of rice bran with pond water and tap water.

Table 2. Mean values of preadult instars (number of animals in parentheses), adult instars, maximum growth increment (number at which instar number in parenthesis), total number of eggs, longevity and size at death of *D. similis* under different experimental conditions.

Experimental conditions	No. of preadult instars	No. of adult instars	Maximum growth increment (mm)	Total no. of eggs	Longevity (days)	Size at death (mm)
Pond water	4(10)	11	0·470(3)	126	20	2·706
Pond water + 250 mg/l rice bran	3(2)–4(8)	18	0·749(2)	429	31	3·065
Pond water + 500 mg/l rice bran	3(3)–4(7)	22	0·722(2)	642	38	3·300
Pond water + 1000 mg/l rice bran	4(10)	20	0·660(2)	658	33	3·777
Tap water	—	—	—	—	1	—
Tap water + 250 mg/l rice bran	3(1)–4(9)	12	0·289(2)	86	23	2·228
Tap water + 500 mg/l rice bran	3(1)–4(9)	10	0·226(1)	8	16	1·914
Tap water + 1000 mg/l rice bran	3(1)–4(9)	8	0·389(2)	34	13	1·965

culture media. The differences in egg production at each instar under different culture conditions are statistically significant for *D. similis* (table 3).

The differences in size of individual at each instar are calculated as growth rate. The mean size of *D. similis* at each instar under different culture conditions were shown in figure 4. From the figure it is observed that the growth rate is exponential at the earlier phase of the life cycle and on the onset of reproduction it showed only a gradual increase.

Table 3. Analysis of variance: Differences in the number of egg production in relation to adult instar number of *D. similis* under different experimental conditions.

Experimental conditions	Degrees of freedom	'F' value	Probability
Pond water + rice bran 250, 500, 1000 mg/l	2, 52	4·789	0·05[a]
Tapwater + rice bran 250, 500, 1000 mg/l	2, 18	12·503	0·005[b]
Pond water + rice bran 250 mg/l, tap water + rice bran 250 mg/l, pond water alone.	2, 35	53·095	0·005[b]
Pond water + rice bran 500 mg/l, tap water + rice bran 500 mg/l, pond water alone.	2, 32	10·872	0·005[b]
Pond water + rice bran 1000 mg/l, tap water + rice bran 1000 mg/l, pond water alone.	2, 36	29·267	0·005[b]

[a] Significant. [b] Highly significant.

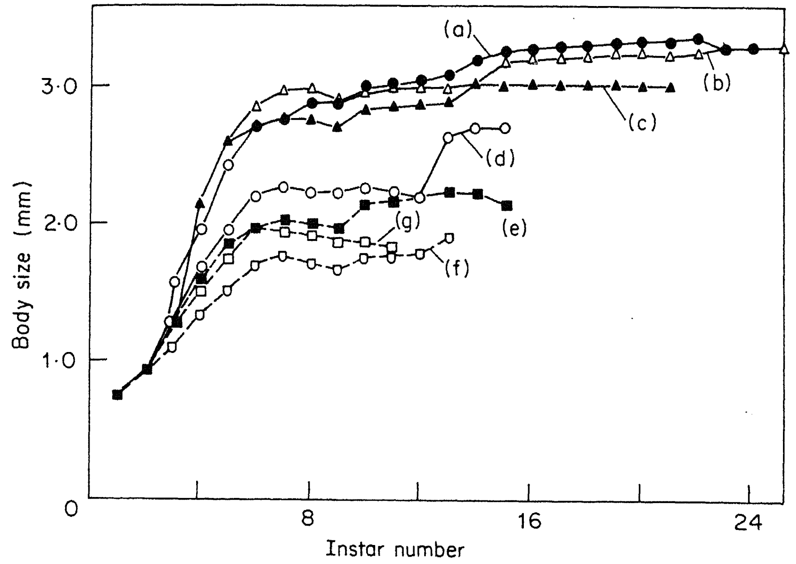

Figure 4. Mean body size in relation to instar number of *D. similis* cultured in different experimental conditions. a. 1000 mg/l rice bran + pond water; b. 500 mg/l rice bran + pond water; c. 250 mg/l rice bran + pond water; d. pond water alone; e. 250 mg/l rice bran + tap water; f. 500 mg/l rice bran + tap water; g. 1000 mg/l rice bran + tap water.

4. Discussion

The observations on longevity show that 50 % of *D. similis* were survived for 10–15 days in tap water with rice bran and 30–35 days in pond water with rice bran (figure 1). Females cultured in plain tap water lived for one day and in plain pond water 20 days. The same animal cultured in rice bran with pond water lived more number of days than in tap water with rice bran and plain tap water or plain pond water (table 2). A review of literature shows that in *D. longispina* (Ingle *et al* 1937), *D. pulex* (Arnold 1971) and in *D. similis* and *D. cephalata* (Venkataraman 1983) the survival values are higher at low food levels. Ingle *et al* (1937) and MacArthur and Baillie (1929) on the other hand reported that longevity was controlled by temperature. But Arnold (1971) found a striking influence of food type on longevity with reference to *D. pulex* at 20°C. He found the highest longevity of animals fed with green algae as food and medium values in medium food concentrations. He also found the lowest values of longevity with blue green algae as food as found by Porter and Orcutt (1980). In the present study, *D. similis* showed a higher life span in 3 different concentrations of rice bran with pond water than in rice bran with tap water (table 2). This type of highest longevity in pond water with rice bran may be due to the quality of the medium which contains other inorganic substances which may be absent in tap water.

It is of interest to compare the number of preadult instars of *D. similis* under different culture media. The results show that the preadult instars vary between 3–4 (table 2). Among daphnids both variable and constant number of preadult instars have been reported by many authors. Anderson (1932) found a constant number of juvenile instars in *D. magna* reared in Banta's manure-soil medium, but a variable number when reared on an oat meal modification of Banta's medium. However, Anderson *et al* (1937) and Anderson and Jenkins (1942) used an unmodified Banta's manure-soil medium and found a variable number of juvenile instars for *D. pulex* and *D. magna*.

The variation in number of eggs produced in a life span of *D. similis* is marked between different culture conditions. The mean total number of eggs produced by *D. similis*, when cultured in pond water with 1000 mg/l of rice bran is 658 in a life span of 33 days (table 2), which is almost 3 times greater than the same species cultured in modified Banta's medium (213·66 eggs in a life span of 37 days) (Venkataraman 1983). Statistical analysis (ANOVA) of the egg production under different experimental conditions shows that the differences are highly significant (table 3). The regression analysis of cumulative number of eggs produced under different experimental conditions shows that the rate of egg production is higher in animals cultured with pond water than in animals cultured with tap water plus rice bran (figure 3).

The number of eggs produced by an adult *Daphnia* of any size depends upon food intake (Hebert 1978). Evidences to support this view among cladocerans have been given by Dunham (1938) and Anderson and Jenkins (1942). Adult females without eggs are common in food limited laboratory populations (George and Edwards 1974). Number of experimental studies (Ingle *et al* 1937; Richman 1958; Hall 1964) have shown that there is a direct relationship between food supply and the number of progeny produced by a female during her life time. However, there is a lower limit to food level below which no egg production occurs and there is also an upper limit above which egg number fails to increase (Richman 1958). Other environmental factors such as oxygen concentration and pH (Fox *et al* 1951; Kring and O'Brien 1976; Ivanova

1969), light regime (Buikema 1973) and temperature (Burns 1969; Kibby 1971; Venkataraman 1983) may cause variation in egg number.

As in other Cladocera, *Daphnia* undergoes a series of moults, growth occurring shortly after each moult. The growth curves of *D. similis* as shown in figure 4 are similar to that of other temperate and tropical cladocerans (Banta *et al* 1939; Richman 1958; Hall 1964; Venkataraman 1981, 1983). In the present study the maximum growth increment occurred between 1–3 instars (table 2). Analysis of the data on life cycle of some tropical Cladocera reveals that maximum growth occurs in the early preadult instars (Murugan and Sivaramakrishnan 1973; Venkataraman 1981, 1983). A sudden decrease in somatic growth after reaching maturity as was found in the present study has also been mentioned by Anderson (1932), Kryutchkova and Sladecek (1969) and Wegelenska (1971). This may be partly an effect of gonad production, since Kryutchkova and Sladecek (1969) showed the adult *Daphnia* used 66–75% of their absorbed energy for egg production.

The size at death of females at different culture conditions vary. The size at death of animals cultured in pond water with rice bran is higher than the animals cultured in tap water (table 2). Hebert (1978) found that the size reached by individuals of a particular species depends upon their food supply and when the food supply is meagre, adults cease to grow on reaching maturity. When the food is abundant growth continues throughout life. Vijverberg (1980) found that the growth is mainly a function of temperature, age, food conditions and maximum size of the species concerned.

Thus from the above mentioned observations it may be concluded that rice bran with pond water increases life span, total number of eggs and size of *D. similis* under laboratory conditions.

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Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca)

N MUNUSWAMY and T SUBRAMONIAM

Unit of Invertebrate Reproduction, Life Sciences Building, Department of Zoology, University of Madras, Guindy Campus, Madras 600 025, India

MS received 20 July 1985; revised 31 December 1985

Abstract. Histochemical characterisation of ooplasm and yolk granules in different stages of oocyte maturation in the ovary was made in the freshwater fairy shrimp *Streptocephalus dichotomus*. In stage I oocyte, the ooplasm is reactive to basic and acidic groups of protein, aromatic groups such as tryptophenyl and tyrosyl and sulphur containing substances. The other organic compounds include acid mucopolysaccharide and acidic lipids. In stage II oocyte, sudanophilic lipoidal substances appear coinciding with yolk formation. Stage III oocytes are characterised by the abundance of yolk granules which are rich in sulphated acid mucopolysaccharide as well as glycogen. Besides these, the presence of acid phosphatase activity in the ooplasm and nurse cell is also noticed and its functional role in relation to vitellogenesis is discussed.

Keywords. Yolk granules; sulphated AMP; auto-heterosynthesis.

1. Introduction

Studies on oogenesis in Crustacea is limited mainly to malacostracans (Blinski 1979; Zerbib 1980; Adiyodi and Subramoniam 1983). During vitellogenesis, various yolk components appear sequentially. For many decapods, yolk is histochemically characterised (Zerbib 1980; Varadarajan and Subramoniam 1980). The present paper reports on the origin of various yolk components, as revealed by histochemical characterization in a freshwater anostracan *Streptocephalus dichotomus* Baird. Ultrastructural aspects of oogenesis in this crustacean is reported elsewhere (Munuswamy and Subramoniam 1985).

2. Materials and methods

The ovaries of mature female *S. dichotomus*, were dissected out and fixed in 5 % neutral buffered formalin for protein and carbohydrates. For lipid, fresh as well as formol calcium fixed tissues were used.

Histochemical procedures to detect and characterize the various yolk components such as proteins, carbohydrates, lipids and the acid and alkaline phosphatase activity were adopted from Lillie (1965), Pearse (1968), Chayen *et al* (1973), Bancroft and Stevens (1975) and Pearse (1972).

3. Results

3.1 *Stage I*

The oocytes gave positivity for protein tests such as *p*-dimethyl amino benzaldehyde and Millon's, indicating the presence of tryptophan and tyrosyl groups respectively.

Acidic and basic groups of protein were markedly abundant in the nucleus and cytoplasm, as revealed by mercuric and aqueous bromophenol blue. Sulphydryl and disulphide groups were also detected by a positive reaction to ferric ferricyanide and performic acid alcian blue with appropriate controls. Presence of DNA in the nucleus and RNA in the cytoplasm was revealed from the staining reactions of Feulgen test and methyl green pyronin G respectively (table 1).

The periodic acid schiff test (PAS) positivity may suggest the presence of a variety of carbohydrate components. The PAS positivity was considerably reduced after the diastase treatment suggesting the presence of glycogen in the cytoplasm.

The positive reaction of PAS after diastase treatment showed the presence of acid mucopolysaccharide (AMP). Further characterisation of AMP with alcian blue and toluidine blue indicated its carboxylated nature (table 2).

The presence of lipid in the cytoplasm of the oocyte was demonstrated by Sudan black B, and oil red 'O'. The appearance of blue colour with Nile blue sulphate indicated the presence of acidic lipids in the cytoplasm, but the negativity to acid haematin suggested the absence of phospholipids (table 3).

3.2 *Stage II*

A gradual decrease in basophilia in the nucleus and later in the ooplasm was evident in stage II, increased staining at this stage with aqueous bromophenol blue shows the basic protein nature of yolk. With Mallory's triple stain, the oogonial cells and the nuclei of the oocytes stain blue, whereas the yolky ooplasm stained orange red, indicating the presence of basic protein.

Ooplasm revealed the presence of basic and acidic groups; tyrosyl, tryptophanyl groups, sulphydryl and disulphide groups. Acid phosphatase was also localised in the ooplasm as well as nurse cells in the second stage of oocyte maturation. Besides this, the follicle cells in the ovarian wall showed intense positivity to acid phosphatase. The histochemical tests revealed the presence of glycogen and AMP in the cytoplasm. In this stage the glycogen appears and continues to accumulate in the cytoplasm during the growth of the egg until yolk granules appear in the oocytes (tables 1 and 2).

Phospholipids formed the prominent lipid component of yolk granules (table 3). The cytoplasm of the stage II oocyte showed high RNA content. Sudanophilic lipoidal substances made their appearance in the yolk granules. Nurse cells which are prominent in the first and second stages of oocyte development showed positive reactions to protein, DNA and RNA tests.

3.3 *Stage III*

In stage III, yolk granules were abundant and the mature oocytes were ready to be released into the lateral pouch of oviduct. Histological stains, haematoxylin eosin and Mallory's triple stain gave eosinophilic and red colour to the yolk granules respectively. The yolk consisted predominantly of basic proteins, rich in aromatic, disulphide and sulphydryl groups (table 1).

Glycogen accumulation in the granules increased with the onset of yolk formation. The histochemical tests with appropriate controls further indicated the presence of sulphate groups and phosphate groups in the AMP. The outer layer of oocyte showed positivity to alkaline phosphatase activity as inferred from Gomori test (table 2).

Coarse sudanophilic lipid granules were characteristic of all growing oocytes. Intensity to acid haematin after the pyridine treatment is of considerable interest because intense staining may be due to the presence of protein rather than lipids. The results of performic acid-schiff and its control revealed the presence of unsaturated fatty acid in the oocytes (table 3).

4. Discussion

The cytochemical observations made on the growing oocytes and nurse cells of *S. dichotomus* have revealed the sequential deposition of different deutoplasmic substances in the oocytes. In early stages of ovarian maturation the ooplasm is devoid of any yolk granule and the cytoplasm shows much affinity for basic dyes such as haematoxylin. This basophilia is gradually replaced by acidophilia in the ooplasm as vitellogenesis advances. Concomittant with this change in the staining property of ooplasm, small glycoprotein granules appear in the perinuclear region. This granulation intensifies further and fills up the entire cytoplasm. The mature granules stain for glycolipoprotein. In *Streptocephalus dichotomus* the granules are of the same type unlike the granules in the oocyte of *Chirocephalopsis bundyi*, where two types have been reported histochemically (Linder 1959). The changing histochemical property of the yolk components has also been reported in many decapod oocytes (Blinski 1979; Zerbib 1980).

Anostracans are ideal crustaceans to study oocyte-nurse cell association during vitellogenesis. The presence of nurse cells in the early phase of oocyte development suggests an initial autosynthetic mode of yolk formation in *S. dichotomus* as the nurse cells supply only RNA at this time. In the brine shrimp *Artemia*, the nurse cells become polyploid by endomitosis and extrude DNA from the nucleus into the cytoplasm before being phagocytosed by the oocyte (Fautrez-Firlefyn 1951). However, the process of vitellogenesis is not purely internal to oocyte. The histochemical observations on the yolk granules of *S. dichotomus* reveal both auto and heterosynthetic mode of yolk formation. This condition may be comparable to an anomuran crab *Clibanarius clibanarius* in which the yolk formation takes place by heterosynthesis to supplement the initial autosynthesis (Varadarajan and Subramoniam 1980).

In addition to the nurse cells RNA contribution to the vitellogenic oocyte comes from the nucleolus. Besides this, presence of basic protein in the nucleoplasm suggest the metabolic role of the nucleolus (Fautrez-Firlefyn and Fautrez 1953).

Interestingly, the localization of acid phosphatases in the stage II oocyte as well as in the follicle cells suggest their involvement in the process of vitellogenesis. Acid phosphatase is an important hydrolytic enzyme mediating the degradation and transfer of nurse cell products to vitellogenic oocyte in a hemipteran insect, *Gerris remigis* (Cone and Eschenberg 1981).

Table 1. Histochemical reactions for proteins in the oocytes during vitellogenesis.

Tests	Stage I		Stage II		Stage III	To detect
	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Yolk globules	
Mercuric bromophenol blue	+ B	+ + DB	+ B	+ + DB	+ + DB	General proteins
Aqueous bromophenol blue (ABB)	+ B	+ B	+ B	+ B	+ + DB	Basic proteins
Deamination + ABB	–	–	–	–	–	Removal of amino groups
Fast green at pH 1	± G	+ G	–	+ + G	+ G	Acidic proteins
Fast green at pH 8	–	± G	–	± G	+ + G	Basic proteins
Toluidine blue (TB)	+ B	+ B	+ B	+ B	+ + B	Acidic proteins
Methylation + TB	–	–	–	–	–	Removal of acidic groups
Ninhydrin	+ B	+ B	+ B	+ B	+ + B	Amino groups
Millon's	–	+ BR	–	+ BR	+ + BR	Tyrosine
Bromination + Millon's	–	–	–	–	–	To block-OH group
p-Dimethyl amino benzaldehyde (DMAB)	–	+ BC	–	+ BC	+ + + BC	Tryptophan
40% Formalin + DMAB	–	–	–	–	–	To block tryptophenyl groups

Ferric ferricyanide (FFC)	–	+ PB	–	+ PB	++ PB	-SH groups
Mercuric chloride + FFC	–	–	–	–	–	To block-SH groups
Performic acid alcian blue (PFAB)	+ B	+ B	+ B	+ B	++ B	
Performic acid Schiff	+ M	+ M	+ M	+ M	+ M	-S-S- groups
Thioglycollate + PFAB	–	–	–	–	–	To block-S-S-groups
Feulgen's	+ M	–	+ M	–	+ M	DNA
Methyl green pyronin G (MGP)	+ G	+ G	+ G	+ G	+ G	RNA
10%Cold perchloric acid + MGP	–	–	–	–	–	To extract RNA
Gomori's	–	±	–	+ Br	++ Br	Acid phosphatase
Gomori's	–	–	–	+ Br	++ Br	Alkaline phosphatase

B, blue; Bb, bluish black; Br, brown; BR, brick red; DB, dark blue; G, green; M, Majenta; Pb, prussiun blue; R, red.
–, absent; ±, doubtful; +, ++, +++, degree of positive reaction intensity.

Table 2. Histochemical reactions for carbohydrates in the oocytes during vitellogenesis in *S. dichotomus*.

Tests	Stage I		Stage II		Stage III	To detect
	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Yolk globules	
Schiff alone	–	–	–	–	–	Free aldehydes
Periodic acid schiff (PAS)	–	+ M	–	+	+ + + M	Glycogen, 1–2 glycol group, unsaturated fatty acid and acid mucopolysaccharide
Diastase + PAS	–	± M	–	+ M	+ + M	Removal of glycogen
Acetylation + PAS	–	± M	–	± M	± M	Removal of 1, 2 glycol group
Deacetylation + PAS	–	+ M	–	+ M	+ M	1,2 glycol group
Bromination + PAS	–	–	–	–	–	To block C-C groups
Best's carmine (BC)	±	+	±	+ +	+ +	Glycogen
Aldehyde fuchsin	–	+ P	–	+ P	+ +	Sulphated and non-sulphated acid muco substances

Alcian blue Critical electrolyte concentration (CEC)						
0.2 M	–	+++ B	+++ B	+++	+	Carboxylated muco substances
0.4 M	–	++	–	++ B	++ B	Phosphated muco substances
0.6 M	–	+++ B	–	++	+++ B	
1.0 M	–	–	–	–	+++ B	Strongly sulphated muco substances
Toluidine blue at different hydrogen ion concentration						
pH 2	–	++	–	++ B	+++ V	Sulphated muco-polysaccharide
pH 3	–	+++ B	–	++ B	+++	
pH 4	–	+++ B	–	+++	++	Phosphated and carboxylated mucosubstances
pH 5	–	+++ B	–	++ B	+ B	Carboxylated substances
Benzidine		–	–	–	++ B	Sulphate group

P, pink; V, violet.

Table 3. Histochemical reactions for lipids in the oocytes during vitellogenesis in *S. dichotomus*.

Tests	Stage I		Stage II		Stage III	To detect
	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Yolk globules	
Sudan black B (SBB)	± Bb	+ Bb	± Bb	+ Bb	+ + Bb	General lipids
Chloroform/methanol + SBB	–	± Bb	–	± Bb	± Bb	Removal of lipids
Hot acetone + SBB	–	± Bb	–	+ Bb	± Bb	Bound lipids
Nile blue sulphate 1% (NBS)	+ B	+ B	+ B	+ B	+ + B	Acidic and neutral lipids
Chloroform/methanol + NBS	–	–	–	–	–	To remove lipids
Oil red 'O'	–	+ R	–	+ R	+ R	Neutral lipids
Acid haematin (AH)	–	+ R	–	+ + R	+ + + R	Phospholipids
Chloroform/methanol + AH	–	–	–	–	–	To remove lipids

For abbreviation and symbols see table 1.

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Holocycly, seasonal activity, morphometry and natural enemies o willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae in the Indian region

D GHOSH, P K MEDDA and S CHAKRABARTI

Biosystematics Research Unit, Department of Zoology, University of Kalyani, Kalyan
741 235, India

MS received 8 July 1985; revised 16 December 1985

Abstract. The aphid, *Cavariella aegopodii* is a holocyclic species, alternating between willo and plants of the family Umbelliferae. Its biology and natural enemies are discussed Morphometry of different morphs are also provided here.

Keywords. Aphid; *Cavariella aegopodii*; morphometry; biology; natural enemies.

1. Introduction

Cavariella aegopodii, a cosmopolitan and polyphagous aphid, is a serious pest of carro (*Daucus carota*) in Australia and France where it transmits 'carrot motley dwarf virus (Eastop 1966; Brunel and Rabasse 1977). In India, however, this aphid has never bee reported on carrot which otherwise harbours two different aphids, viz., *Hyadaphi coriandri* and *Myzus persicae* (Raychaudhuri 1983). In fact, carrot is not usuall cultivated in the mountainous areas where the aphid occurs on willow. Hilly people however, cultivate wild carrot (*Chaerophyllum villosum*) which is so far resistant t aphid infestation.

C. aegopodii, essentially a holocyclic species, leads a heteroecious life on willow an plants of the family Umbelliferae (in New Zealand: Cottier 1935; Middle East Bodenheimer and Swirski 1957; Australia: Eastop 1966; Poland: Szelegiewicz 1968 North America: Stroyan 1969; Japan: Higuchi and Miyazaki 1969; Scotland: Stroya 1976). The present study has been conducted in Garhwal range of western Himalaya a Joshimath (ca. 1900 m) to obtain an account of the biology and natural enemies of thi aphid.

In this paper, morphometric measurements of the different available morphs seasonal and sexual activities, host-association and natural enemies of this aphid hav been discussed.

2. Materials and methods

At Joshimath (Field station in Garhwal range of western Himalaya), several willow (*Salix tetrasperma*) were selected for study. Condition of the plants and occurrence an activity of the aphid (*C. aegopodii*) were observed in natural condition from time to tim during the period September, 1982 to April, 1985. Aphid samples from these plant were collected as and when required and studied. The aphids on the secondary host plants were observed in a bed of Umbelliferae.

3. Observations and discussion

3.1 *Host association and seasonal activities*

3.1.1 *On primary host:* In the area of the present study, 3 species of willows, viz., *Salix tetrasperma*, *S. elegans* and *S. babylonica* are found to occur. This aphid infests the first one, which is wild and distributed on the hill slopes and used as fodder and fuel by hilly people. Besides, the bark of *S. tetrasperma* is used for tanning and also said to be febrifuge (medicinal value) (Watt 1972).

On this plant, this aphid occurs in 2 phases, viz: (i) autumn and winter phase and (ii) spring phase.

3.1.2 *Autumn and winter phase:* Alatae from the secondary hosts immigrate to willows towards October and November (8–28th October in 1982 and 1983; 10th November in 1984). They parthenogenetically produce nymphs which develop into oviparae. They live both on dorsal and ventral surface of leaves. When oviparous colonies are well-established, the alate males make their appearance on the primary hosts (23rd November in 1982 and 2nd December in 1984). It should be mentioned that these males are developed on the secondary hosts only. The ovipara usually remains settled on a particular site of leaf and waits for the mating partner. After the encounter, male approaches towards the back of the female, moves its antennae for a few seconds, slowly mounts over the back of the female. The female raises its caudal portion. Male bends down its abdomen and inserts the aedaegus inside the genital aperture of the female. Mating lasts for about 3–4 min. At the end, male moves sidewise, retracts the aedaegus and moves away. Female remains passive throughout the mating period. A female having eggs in the abdomen is also found to mate for the second time. It appears that it is the male which has the habit of multiple copulation, initiates the second copulation. The male usually dies after 6–7 days of mating.

A fertilised ovipara after 8–10 days of mating starts laying eggs at the buds. The number of eggs produced by a female is 3–4, which are laid during a period of 1–2 days. Each egg is elongatedly oval and orange-yellowish when fresh, but becomes darker afterwards, measuring about 0·6–0·7 mm in length and 0·2–0·3 mm in maximum width. Sometimes 2 females are observed to stay in an oviposition site and thus upto 6 eggs are found in a bud. Ovipara dies after 5–6 days of egg-laying. Eggs are left at this stage for overwintering. All these phenomena are completed before the middle of December (10th December in 1982 and 8th December in 1983), when the leaves of the willow trees gradually turn yellowish. This follows shedding of leaves and the plants remain bare till the end of February.

3.1.3 *Spring phase:* With the onset of spring towards the middle of February, the overwintered eggs hatch into greyish nymphal fundatrices, when the leaves are yet to appear. As a result, the nymphal fundatrices feed on stem-branches and later migrate to sprouting leaves to grow into adult forms which we have missed to collect. They parthenogenetically give rise to nymphal colony of apterous fundatrigeniae, which take

11–12 days for attaining adulthood and lay about 22–28 nymphs during a period of about 14 days with 0–3 nymphs per day. About 3–4 such generations occur on willow, where the alates begin to appear in the 3rd or 4th generations. These alates emigrate to the secondary host during the middle of April (19th April in 1984).

3.1.4 *On secondary host*

As mentioned earlier, plants belonging to the family Umbelliferae constitute the major secondary hosts of this species. Cottier (1953), Eastop (1966), Miyazaki (1971) and Knowlton (1983) reported a number of umbellifer crops and weeds besides a few other hosts from different parts of the world. In India, this aphid has been collected from the following hosts: ***Heracleum soshoweri*** (Raychaudhuri 1980), ***Heracleum*** sp. (Chakrabarti *et al* 1972), ***Pimpinella diversifolia*** (Chowdhuri *et al* 1969; Raychaudhuri 1980) and ***Sanicula europea*** (Raychaudhuri 1980).

The reports of a few other hosts of the family Euphorbiaceae, Polygonaceae, Zingiberaceae (Basu *et al* 1972, 1973; Raychaudhuri *et al* 1980) may be of doubtful occurrence.

In Garhwal range of western Himalaya, from April onwards, alate viviparae (greyish in colour) from the primary host plants begin to emigrate and appear on ***Pimpinella diversifolia*** where they settle on the apical stem or on the inflorescence stalks. These alatae give rise to parthenogenetic viviparous females. Several overlapping generations appear throughout the summer till early autumn. Morphs produced on this plant are apterae and occasionally alatae for migration or to avoid overcrowding. These are dull green in colour matching with the site of infestation. Again, with the onset of autumn (24th October in 1983), alate production in the colony becomes prevalent for immigration to primary host plants. However, it is known that this species can overwinter in Auckland in the viviparous forms on carrot foliage (Cottier 1953).

3.2 *Morphometry*

Measurements of different available morphs of the species indicate that apterae born on secondary host have smaller body in comparison to those on primary host. Alatae produced earlier on secondary host to avoid overcrowding differ from those produced for immigrating to primary host in autumn having smaller body and lesser number of rhinaria (20–28 against 33–36) on the antennal segment III. Again, these immigrating alatae are similar to those born on primary host in spring except for having larger number of rhinaria (33–36 against 16–19) on antennal segment III. The details of morphometry have been provided in table 1.

3.3 *Natural enemies*

Insects belonging to Coleoptera and Diptera comprise the predatory complex of this aphid at least in western Himalaya and also in India, because this report indicates the first finding of natural enemies of this aphid from India. Aphidophagous coccinellid beetles have never been observed to reproduce on this aphid. In spring, these beetles, rather migrate from their breeding sites, somewhere in the herbages and crops, for

Table 1. Morphometric measurements (in mm) of different morphs.

Morphs	Body length	Antennal length	Urs	Ht2	Siphunculi	Cauda	Secondary rhinaria on antennal segments III	IV	V	VI
Primary host:										
Apterae	1·97–1·98	0·77–0·79	0·11–0·12	0·11–0·12	0·34–0·37	0·16–0·17	0	0	0	0
Alatae	2·10–2·16	1·12–1·18	0·10–0·11	0·10–0·11	0·31–0·33	0·14–0·15	16–19	0	0	0
Alate males	1·86–1·87	1·22–1·26	0·11–0·12	0·10–0·11	0·24–0·25	0·10–0·12	40–44	7–12	5–6	3
Oviparae	2·14–2·26	0·69–0·77	0·12–0·13	0·10–0·11	0·31–0·37	0·14–0·16	0	0	0	0
Secondary host:										
Apterae	1·24–1·34	0·52–0·58	0·08	0·08	0·23–0·29	0·11–0·12	0	0	0	0
Alatae	1·76–2·12	1·10–1·21	0·10–0·11	0·10–0·11	0·30–0·32	0·14–0·15	20–36	0	0	0

Apterae, Apterous viviparous females; Alatae, Alate viviparous females; Oviparae, Apterous oviparous females; Urs, Ultimate rostral segment; Ht2, Second segment of hind tarsus.

exploitation to all available food sources. The coccinellids include *Coccinella septempunctata*, *Oenopia kirby* and *O. sauzeti* on primary host and *Menochilus sexmaculatus* and *O. sauzeti* on secondary host. *Metasyrphus confrater* is the only syrphid species collected from willow.

Regarding parasites, though Takada and Rishi (1980) reported *Ephedrus minor* as the aphidiid parasite of *Cavariella* spp. from *Salix*, no such collection was made in the present work both on primary and secondary hosts. However, in France, Rabasse and Brunel (1977) collected *Aphidius salicis* as the primary parasite of *C. aegopodii* on carrot besides *Ephedrus salicicola* and *Trioxys* (*Binodoxys*) *heraclei*. Narayanan *et al* (1962) in their world catalogue of aphidiine parasite, mentioned *Ephedrus plagiater* as the only aphidiid parasite of *C. aegopodii*.

Acknowledgements

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Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae)

B CHANDRIKA and N K BALASUBRAMONIAN

Department of Aquatic Biology and Fisheries, University of Kerala, Trivandrum 695 007, India

MS received 26 August 1985

Abstract. The length-weight relationship of *Xenentodon cancila* (Ham.) was calculated for males and females. A total of 416 females and 141 males ranging from 101–230 mm in total length was used for the study. Results showed that there is a close relationship between the length and weight of fish. Significant difference was found between the regression coefficients of males and females of *Xenentodon cancila*. By testing the regression coefficients against the isometric growth values of 3, it was seen that for both males and females, the growth pattern agrees with the isometric growth formula.

Keywords. *Xenentodon cancila*; analysis of covariance; length-weight relationship; 't' test.

1. Introduction

While attempting a study of the biology of a fish, it is usual to analyse the mathematical relationship between its length and weight. This analysis will reveal the extent to which the two variables, length and weight, are related to each other, and thereby help one to calculate with ease one variable when the other is known.

In the natural habitat, the weight of a fish increases as the length increases, thereby showing that the weight of a fish is a function of length. Since length is a linear measurement and weight a measure of volume, it was generally found that, for fishes the relation between length and weight could be expressed by the hypothetical cube law,

$$W = CL^3,$$

where W represents the weight of the fish, L its length and C a constant. This cubic relationship holds good only in the ideal fish where the specific gravity and form remain unaltered as they grow. As pointed out by Le Cren (1951) since fishes normally do not remain the same shape or body outline throughout their life time and since the specific gravity of the tissues also may not remain constant, the actual relationship may sometimes depart significantly from this cubic relationship. In such fishes it is better to fit a general parabolic equation,

$$W = aL^n$$

where W represents the weight of the fish, L its length, a a constant equivalent to C and n a constant to be calculated empirically from the data. This relationship is usually expressed in its logarithmic form:

$$\text{Log}\, W = \log a + n \log L.$$

Xenentodon cancila forms an important minor fishery of Kerala state and details regarding its growth rates are entirely lacking. Hence an attempt was made to study the length-weight relationship in this species.

2. Material and methods

A total of 557 specimens of *X. cancila*, 416 females and 141 males ranging from 101–230 mm in standard length collected during April 1980–March 1981 were used for the present study. All the specimens were procured from the Vellayani freshwater lake in Trivandrum. For each specimen, the standard length in mm was measured from the tip of the snout to the base of the caudal fin and the weight was taken correct to mg in a chemical balance.

In order to find out whether the two sexes of *X. cancila* showed any difference in the length-weight relationship, analysis of covariance was employed (Snedecor and Cochran 1975). The relationship thus estimated was represented graphically to get an idea of the relation between the two variables, weight and length, and also the extent of variation, if any between the sexes. Prior to analysis, the values of W and L were converted to their logarithms, thus the length-weight relationship becomes $\log W = \log a + n \log L$.

3. Results and discussion

Table 1 represents the analysis of covariance to test if the regression coefficients are significantly different for the two sexes of *X. cancila*. It was found that the regression coefficients of the males and females were significant at 1% level, hence separate equations were worked out for the sexes. The relationship between the length and weight was therefore fitted separatedly for the two sexes which is as follows:

1. Females $Y = 2{\cdot}8333\, x - 5{\cdot}1918$
2. Males $Y = 2{\cdot}9014\, x - 5{\cdot}3470$.

Table 1. Analysis of covariance for testing differences in regression coefficients between males and females of *X. cancila* (Ham.)

Source of variation	Degree of freedom	Sum of squares	Mean of sum of squares
Females	414	3·1478	0·0076
Males	139	1·3061	0·0094
	553	4·4539	0·0081
Pooled within	554	4·4552	0·0080
Difference between slopes	1	0·0013	0·0013
Within + between	555	4·7637	0·0086
Difference between adjusted means	1	0·3085	0·3085

For comparison of slopes: Fs = 0·1621.
For comparison of elevations: Fe = 38·5625 (significant at 1% level).

The graphical representations of the same are presented in figures 1 and 2.

In the cube law $W = CL^3$, the value of the exponent n will be 3. For an ideal fish which maintains a constant shape, the value of n will be 3 (Allen 1938). While according to Hile (1936) and Martin (1949) the value of n lies between 2·5 and 4. Beverton and

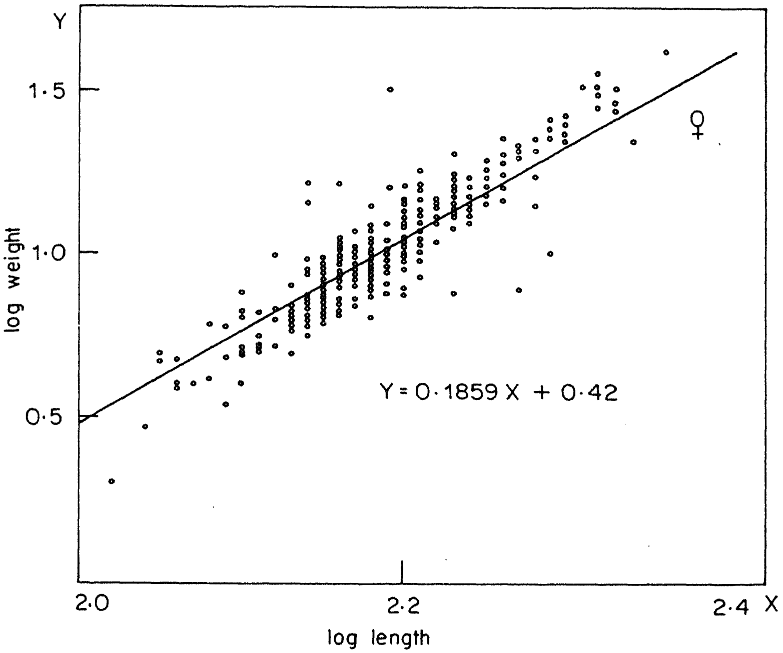

Figure 1. Length-weight relationship of females.

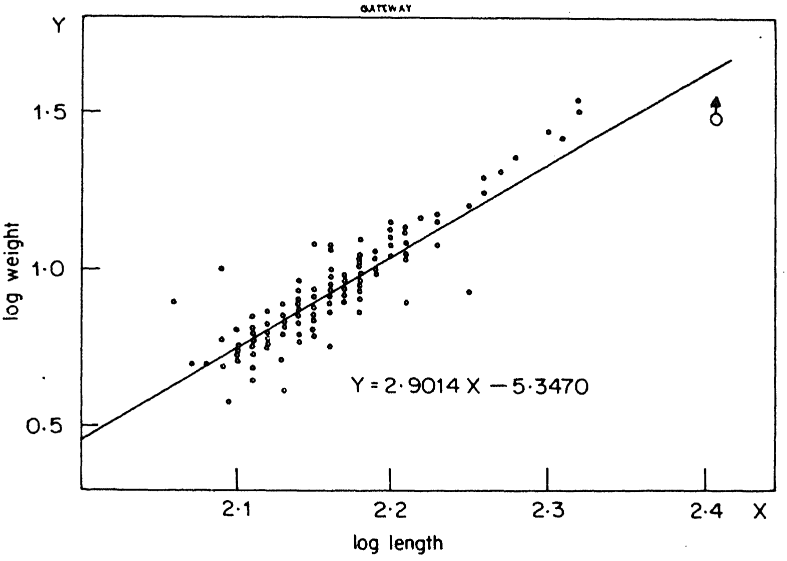

Figure 2. Length-weight relationship of males.

Holt (1957) are of the view that important departures from isometric growth in adult fishes are rare. In order to determine whether any significant variation occurred from the expected value of 3·0 for the ideal fish in the estimate of B for the female and the male of *X. cancila*, '*t*' test was applied using the formula:

$$t = \frac{|3.0 - B|}{S_B},$$

where S_B is the standard error of the estimate of B. The corresponding t values were 0·7569 and 0·3719 respectively for the female and male showing that in both cases the estimate of B is not significantly different from 3.0. Hence the cubic formula $W = CL^3$ would be a proper representation of the length-weight relation of both females and males of *X. cancila*. Both the sexes do not show any significant departure from the isometric growth pattern.

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Wings and wing-microsculpturing in the termite family Indotermitidae (*Indotermes*, Isoptera), and their bearing on phylogeny

M L ROONWAL and N S RATHORE

Desert Regional Station, Zoological Survey of India, Paota B Road, Jodhpur 342 006, India

MS received 9 September 1985

Abstract. The wings and wing-microsculpturing, hitherto either unknown or poorly known, in the small, rare family Indotermitidae, is described here in imagoes of *Indotermes rongrensis*. Wings are transparent, colourless and weakly hairy; wing venation is described. Microsculpturing on wings consists of a row of small, pointed papillae at the anterior margin and a dense carpet (3100–3800/mm^2) of minute (1–6 μm) simple, nonasteroid micrasters on both the membranes. The phylogeny of the Indotermitidae is discussed. It arose as a lone branch (with 3-segmented tarsi) from the initially 4-segmented ancient Termitidae stock.

Keywords. Wings; wing-microsculpturing; Indotermitidae.

1. Introduction

Wing-microsculpturing in termites has recently been extensively studied by Roonwal and co-workers (1967–1983, summary in Roonwal 1983) in all the families except two small ones. A dense carpeting (densities up to over 12500/mm^2) of minute, cuticular structures of at least 8 different types have been found on both the upper and lower wing surfaces. The two exceptional, rare and single-genus families are the Serritermitidae (*Serritermes*) from South America and the Indotermitidae (*Indotermes*) from South-East Asia. The former has recently been studied (Roonwal and Rathore 1985) and results on the latter are presented here.

2. Results

Winged imagoes of a single species were available.

Indotermes rongrensis (Roonwal and Chhotani 1962)

[*Speculitermes cyclops rongrensis* Roonwal and Chhotani 1962, p. 314; *S. rongrensis*, Roonwal and Chhotani, 1977, p. 50; *Indotermes pakistanicus* Chaudhry and Ahmad 1972, p. 42, nom. nudum; *Indotermes bangladeshiensis* Akhtar 1975, p. 116.]

Material Imagoes from the Dawki Road from Pynursila to Pomshutia, ca. 25-15 N lat., 9-50 E long. (Meghalaya, NE India).

2.1 *Wings* (*figure* 1)

Wings of the family Indotermitidae are poorly known and have never been illustrated except for a sketchy figure (Tsai and Chen 1963) of a Chinese species, *I. isodentatus*. The wings of *I. rongrensis* are, therefore, described below in some detail.

Wings transparent and colourless, scale and base dark brown; veins faint except the two long anterior ones. Hairs on membrane few, colourless and small (length 40–80 μm), longer (90–210 μm) on the scales; 3–4 rows on the anterior margin (costa), a row on the radius, 1–2 rows on the posterior margin; and sparse or almost absent on the membrane except on the distal fifth where they are more numerous (figure 5). Forewing: Length with scale 13–16 mm, maximum width 3·3 mm; forewing scale ca. 1·0–1·1 mm long. Of the veins, C long, thick, well chitinised; Sc short, joining C just outside the scale; R long, thick, running close to and parallel to C; M arising independently and with 3–4 branches; Cu with 8–12 branches. Hindwing: Length with scale 12·0–14·5 mm, maximum width 3·5 mm; scale 0·7–0·8 mm long. C and R as in forewing; Sc absent; M arising from R and with 3–4 branches; Cu with 10–12 branches. In an earlier account, Roonwal and Chhotani (1977), the short subcosta was regarded as the radius and the present radius as the radial sector. We believe that the present interpretation is the more appropriate one and is in consonance with the comparative condition in other termites.

2.2 *Wing-microsculpturing* (*figures* 2–5)

Microsculpturing consists of papillae and micrasters on both wing surfaces, as follows:

Papillae: A row of minute (1–3 μm long) papillae on the anterior margin; none on the posterior.

Micrasters: Minute (1–6 μm), simple and nonasteroid, with 1–3 arms (rods, length 4–6 μm, width 1 μm; V-shaped, 2–4 × 2–3 μm; Y-shaped, 3–4 × 2–3 μm). Rods are the

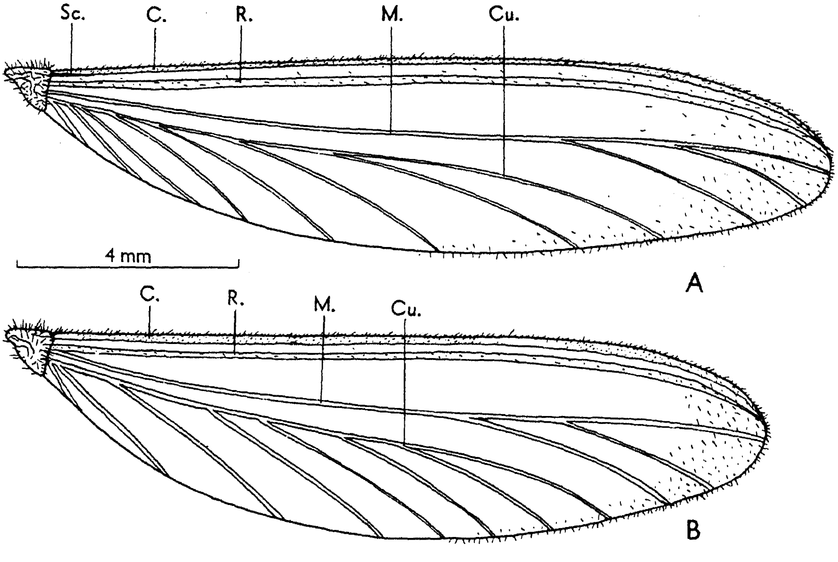

Figure 1. *Indotermes rongrensis.* Wings of right side to show the veins and the distribution of hairs. **A.** Forewing. **B.** Hindwing. C, Costa.; Cu, cubitus; M, media; R, radius; Sc, subcosta.

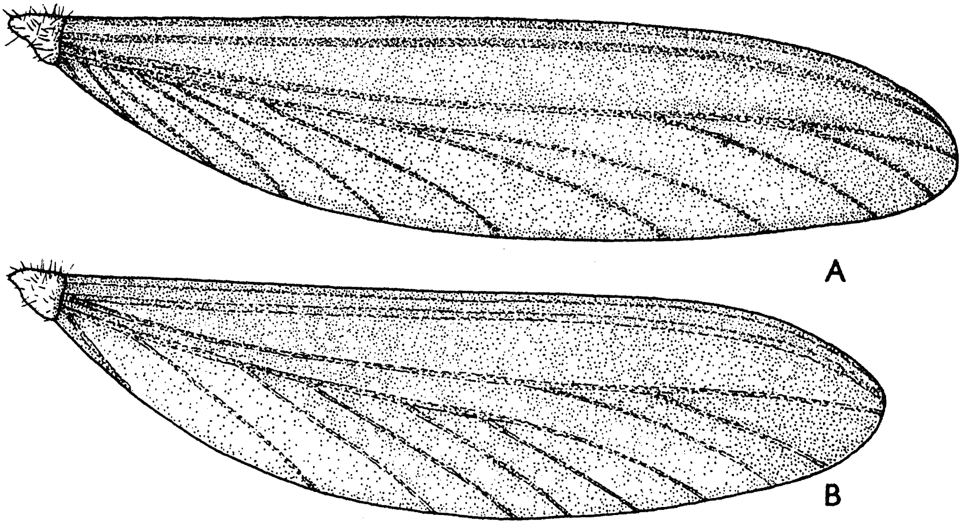

Figure 2. *Indotermes rongrensis.* Outlines of right wings, to show the patchy distribution (stippling) of micrasters on the membrane. **A.** Forewing. **B.** Hindwing.

most numerous, the proportions being: rods 61 %, V-shaped 29 %, Y-shaped 10 %. Widely distributed all over membrane, especially in the distal half, but few or absent in some patches. Density ca. 3100–3800/mm^2, higher in the distal half than elsewhere. In the earlier accounts of Roonwal and Chhotani (1977) and Roonwal and Verma (1980) (under "*Speculitermes rongrensis*"), the Y-shaped micrasters and the patchy distribution were not mentioned.

2.3 *Phylogeny* (*figure* 6)

The family Indotermitidae had been regarded by Roonwal and Sen-Sarma (1960) as being close to the Termitidae, but differing in the following important respects in the soldier caste: (i) Pronotum strongly saddle-shaped (versus either flat or only moderately saddle-shaped); and (ii) tarsi 3-jointed (versus 4-jointed). Initially, only the soldiers were found but now the other two castes (workers and imagoes) are also available. We are thus able to examine the family's status and phylogeny with greater certainty.

The contention of Ahmad (1963) and Krishna (1965) that Indotermitidae cannot be separated, in family status, from the primitive Termitidae (e.g., *Speculitermes*, Amitermitinae) merely on the basis of 3-segmented tarsi, is untenable. Both these authors fell into the curious error of regarding the *Speculitermes* tarsi also as 3-segmented, whereas in all the known species of this genus they are 4-segmented (vide also Roonwal and Chhotani 1966). There are many other points of difference (listed by Roonwal 1975) to which we may now add the characters from wings and their microsculpturing as given in the present account. In the Indotermitidae the wings have few hairs on the membrane and microsculpturing consists of minute, simple micrasters;

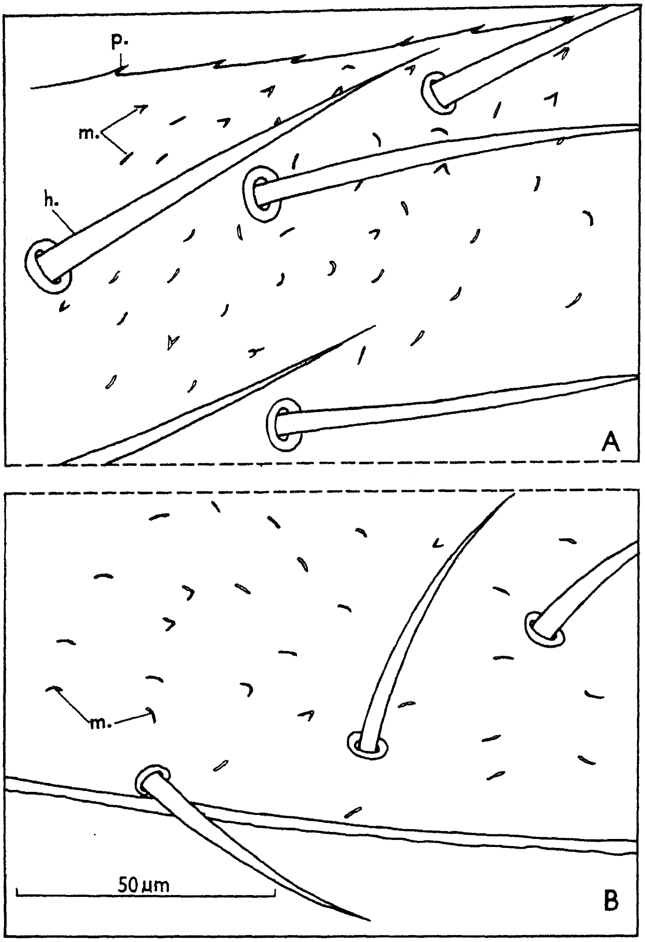

Figure 3. *Indotermes rongrensis.* Dorsal surface of right forewing, to show microsculpturing. **A.** Middle of anterior margin. **B.** Middle of posterior margin. h., hairs; m., micrasters; p., papillae.

in *Speculitermes* (and other primitive Amitermitinae) the wings are more hairy; and the micrasters are much larger, and, though nonasteroid, they have a much greater range of variation (table 1).

The Indotermitidae may thus be visualised as having arisen as a lone subranch (with 3-segmented tarsi) from one of the two principal branches (with 4-segmented tarsi) which arose from the Ancient Termitidae stock (also initially 4-segmented) in the manner depicted in figure 6.

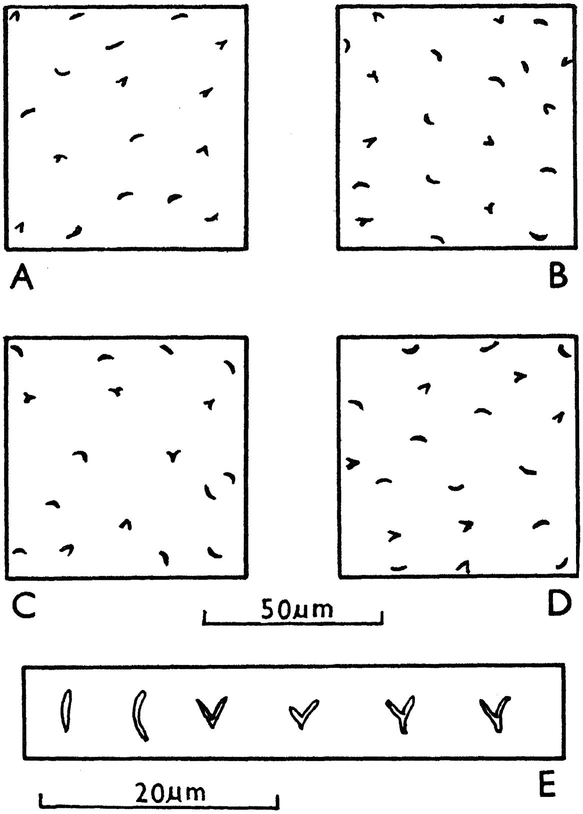

Figure 4. *Indotermes rongrensis.* Dorsal surface of right wing membrane to show microsculpturing (simple micrasters). **A.** Forewing, middle portion. **B.** Same, distal end. **C.** Hindwing, middle part. **D.** Same, distal end. **E.** Types of micrasters, more magnified and rearranged.

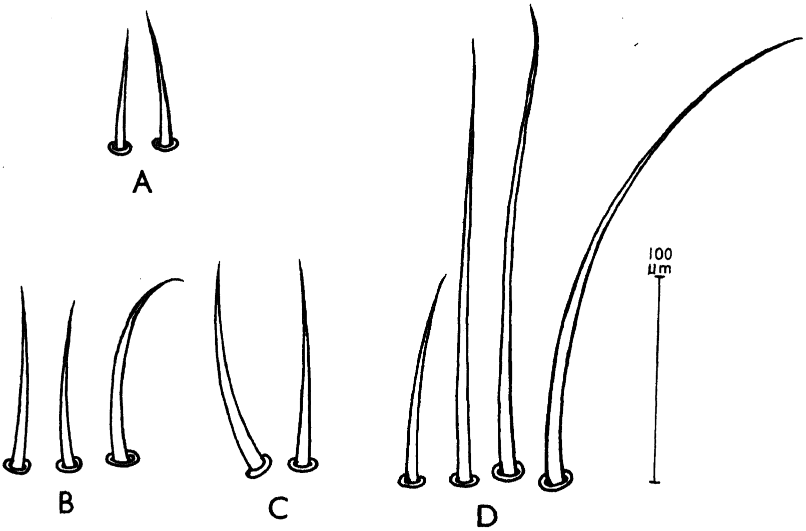

Figure 5. *Indotermes rongrensis.* Hairs on forewing surface. **A.** On membrane (very small, length 40–50 μm). **B.** On distal margin (70–80 μm). **C.** Middle of anterior margin (70–110 μm). **D.** On wing scale (90–210 μm).

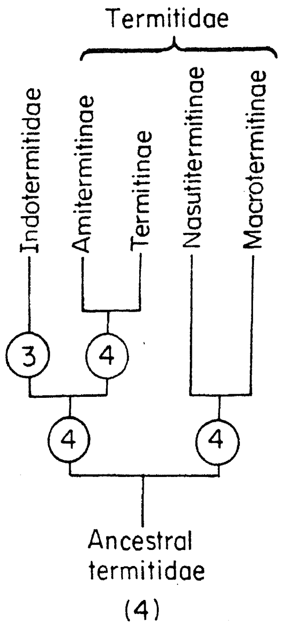

Figure 6. Chart to depict the phylogeny of the Indotermitidae.

Table 1. Comparison of imagoes of *Indotermes* and *Speculitermes*.

Characters	*Indotermes* (Indotermitidae)	*Speculitermes* (Termitidae, Amitermitinae)
Head	Hypognathus	Not hypognathus
Antennae	With 15 segments	With 15–16 segments (mostly 15)
Pronotum	Weakly saddle-shaped; sides strongly narrowing posteriorly	Almost flat; sides subparallel
Wings	With few hairs on membrane	With numerous small hairs on membrane
Wing micro-sculpturing	Wings covered with small papillae (at the margins) and simple, nonasteroid micrasters (with 1–3 arms, mostly 1) which are minute (1–6 μm)	Wings covered with small papillae (at the margins) and simple, nonasteroid micrasters (with up to 4 arms) which are thick and large (5–12 μm)
Legs	Tarsi 3-segmented Apical tibial spur formula 2:2:2 (the third or dorsal spur on foreleg absent)	Tarsi 4-segmented. Apical tibial spur formula 3:2:2 (the third or dorsal spur on foreleg present)

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Competition studies in *Drosophila*: Relative fitness and adaptedness of six closely related species

H A RANGANATH and N B KRISHNAMURTHY

Department of Post-Graduate Studies and Research in Zoology, University of Mysore, Manasa Gangotri, Mysore 570 006, India

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Abstract. Inter and intra specific competition experiments involving 6 taxonomically closely related forms of *Drosophila* have been made. Relative fitness and adaptedness of these forms have shown different levels indicating a lack of correlation between taxonomic proximity and ecological phenotype.

Keywords. Drosophila; competition; fitness.

1. Introduction

'As the species of the same genus usually have, though by no means invariably, much similarity in habits and constitution and always in structure, the struggle will be more between them, if they come into competition with each other than between the species of distinct genera' (Darwin 1859). The understanding of the competitive relationships between closely related species and its appreciation is of considerable evolutionary importance (Parsons 1973). In view of this, competition experiments have been made utilising taxonomically closely related species of the *immigrans* species group of the genus *Drosophila*. The parameters, namely their relative fitness and adaptedness, assessed in the present experiments are presented.

2. Materials and methods

For the present study, 6 species of the *immigrans* species group of *Drosophila* have been employed. They are *D. nasuta nasuta* (Coorg, Karnataka, India), *D.n. albomicana* (University of Texas, USA, collections 3045.11), *D. sulfurigaster neonasuta* (Coorg, Karnataka, India), *D.s. bilimbata* (University of Texas, USA, collections, 3071.6), *D. pulaua* (University of Texas, USA, collections 3121.5) and *D. hypocausta* (Coorg, Karnataka, India).

2.1 *Mixed cultures*

D. n. nasuta was made to compete separately with *D.s. neonasuta, D.s. bilimbata, D. pulaua* and *D. hypoucausta*; similarly, *D.s. neonasuta* was made to compete separately with *D.n. nasuta, D.n. albomicana* and *D. hypocausta.*

Each mixed culture was built with 12 females and 13 males of each species. Four replicates were maintained for each mixed culture. The cultures were maintained at 21°C by the serial transfer technique of Ayala (1965). These mixed cultures were maintained till the elimination of one of the competing species.

The competitive fitnesses of the competing species were calculated by adopting the statistical procedure of Ayala (1969a). If the number of each species change at a constant rate, the fitness of one species relative to the other can be measured by two statistics m and W. The statistic m or logarithm relative fitness is estimated by the coefficient regression of the natural logarithm of the ratio of the numbers of the two species on time (in weeks). The relative fitness, W, is the antilogarithm (to the base e) of m. If m_1 and m_2 measure the fitness of the two strains of one species or of two different species, the fitness of one relative to the other is given by $m_1 - m_2$. The method allows the measurement of both the fitness of one species relative to another and the fitness of strains of the same species relative to each other.

2.2 *Pure cultures*

For all the 6 species under study, single species populations were started with 25 pairs of flies. For each species 4 replicates were maintained at 21°C by adopting the serial transfer technique of Ayala (1965). These pure cultures were maintained for 30 weeks. The mean values for productivity and population size (two parameters of adaptedness) were calculated from 7th week onwards to the 30th week (24 counts).

3. Results and discussion

The interspecific competitive fitness of *D.n. nasuta* was measured during its competition with *D.s. neonasuta*, *D.s. bilimbata*, *D. pulaua* and *D. hypocausta*. In each of these mixed cultures, *D.n. nasuta* was found to eliminate its competitor. *D.s. bilimbata*, *D. pulaua* and *D. hypocausta* were eliminated rapidly within a period of 8 weeks. Particularly, *D.s. bilimbata* was eliminated within two weeks and hence it has not been considered for calculations. *D.s. neonasuta* was the only competitor to survive for a period of 28 weeks and was able to reproduce before it was eliminated by *D.n. nasuta*. The logarithm fitness m and the fitness, W, *of D.n. nasuta* relative to the other 3 species are given in table 1. The logarithm fitness is greater than zero and the relative fitness is more than one in all cases. Analysis of variance for the data in table 1 indicates significant heterogeneity among the 3 kinds of populations. A test for heterogeneity among replicates was made using as mean square error the mean variance of 16 populations. The replicates of the mixed culture *D.n. nasuta*/*D.s. neonasuta* are homogeneous, while others are heterogeneous. The fitness of *D.n. nasuta* with these 3 species, namely *D.s. neonasuta*, *D. pulaua* and *D. hypocausta*, relative to each other are given in table 2. *D.n. nasuta* has evinced maximum interspecific competitive fitness against *D. pulaua* and least against *D.s. neonasuta*.

Similarly, *D.s. neonasuta* was made to compete separately with *D.n. nasuta*, *D.n. albomicana* and *D. hypocausta*. *D.s. neonasuta* eliminates both *D.n. albomicana* and *D. hypocausta* but itself faces termination against *D.n. nasuta*. The logarithm fitness m and fitness, W, for the 12 populations of *D.s. neonasuta* are given in table 3. The analysis of variance for the data in table 3 indicates significant heterogeneity among the 3 kinds of populations. Replicates of *D.s. neonasuta*/ *D.n. albomicana* and *D.s. neonasuta*/*D.*

Table 1. Relative fitness (*W*) and logarithm relative fitness (*m*) of *D.n. nasuta* relative to *D.s. neonasuta*, *D. pulaua* and *D. hypocausta* in 12 experimental populations. The error and the variance of *m* are also given.

Populations	Species	*m* ± SE	Variance	*W*
1.	*D.n. nasuta/D.s. neonasuta*	0·0710 ± 0·020	0·0004	1·074
2.	”	0·0475 ± 0·017	0·0003	1·048
3.	”	0·0274 ± 0·020	0·0004	1·027
4.	”	0·0109 ± 0·020	0·0004	1·011
	Average	0·0392 ± 0·009	0·00009	1·040
5.	*D.n. nasuta/D. pulaua*	0·5556 ± 0·069	0·0048	1·743
6.	”	0·3921 ± 0·126	0·0159	1·480
7.	”	0·4277 ± 0·050	0·0025	1·534
8.	”	0·4584 ± 0·094	0·0089	1·581
	Average	0·4584 ± 0·045	0·0020	1·581
9.	*D.n. nasuta/D. hypocausta*	0·2400 ± 0·065	0·0042	1·272
10.	”	0·2576 ± 0·126	0·0158	1·293
11.	”	0·3256 ± 0·081	0·0066	1·385
12.	”	0·2340 ± 0·086	0·0074	1·264
	Average	0·2643 ± 0·046	0·0021	1·302

Table 2. Comparisons of fitness (*W*) and logarithm fitness (*m*) of *D.n. nasuta* as measured by the competition with *D.s. neonasuta*, *D. pulaua* and *D. hypocausta*.

Species	*m* ± SE	*W*	*P*
D.n. nasuta/D.s. neonasuta *D.n. nasuta/D. pulaua*	−0·4192 ± 0·032	0·6575	< 0·01
D.n. nasuta/D.s. neonasuta *D.n. nasuta/D. hypocausta*	−0·2251 ± 0·032	0·7984	< 0·01
D.n. nasuta/D. pulaua *D.n. nasuta/D. hypocausta*	+ 0·1941 ± 0·032	1·214	< 0·01

hypocausta are heterogeneous. The fitness of *D.s. neonasuta* with these 3 species, viz, *D.n. nasuta*, *D.n. albomicana* and *D. hypocausta*, relative to each other are given in table 4. The differences in fitness values between any two are strikingly significant, demonstrating that *D.s. neonasuta* has evinced different fitness values against its competitors. This indicates differences in the nature of competitive interactions in the mixed cultures of these species.

The intraspecific competitive fitness or the adaptedness of all 6 species is given in table 5. Adaptedness refers to the ability of the carriers of a genotype or a group of genotypes to survive and reproduce in a given environment (Dobzhansky 1968). Two related measures of adaptedness can be obtained, viz, 'productivity' and 'population size' in the experimental populations (Ayala 1965). This provides means for comparing the overall biological performance of one gene pool with another, where both are maintained under similar environmental conditions. Differences in productivity or population size between any two species under study are in most cases significant.

Table 3. Relative fitness (*W*) and logarithm relative fitness (*m*) of *D.s. neonasuta* relative to *D.n. nasuta*, *D.n. albomicana* and *D. hypocausta* in 12 experimental populations. The standard error and the variance of *m* are also given.

Populations	Species	m ± SE	Variance	W
13	*D.s. neonasuta/D.n. nasuta*	−0·0710 ± 0·020	0·0004	0·9315
14	,,	−0·0475 ± 0·017	0·0003	0·9537
15	,,	−0·0274 ± 0·020	0·0004	0·9729
16	,,	−0·0109 ± 0·020	0·0004	0·9893
	Average	−0·0392 ± 0·009	0·00009	0·9616
17	*D.s. neonasuta/D.n. albomicana*	0·2389 ± 0·157	0·0248	1·270
18	,,	0·0977 ± 0·079	0·0063	1·103
19	,,	0·0644 ± 0·046	0·0021	1·066
20	,,	0·0690 ± 0·040	0·0016	1·071
	Average	0·1175 ± 0·046	0·0021	1·123
21	*D.s. neonasuta/D. hypocausta*	0·5222 ± 0·162	0·0264	1·686
22	,,	0·4556 ± 0·082	0·0068	1·573
23	,,	0·3581 ± 0·079	0·0062	1·431
24	,,	0·3193 ± 0·046	0·0021	1·376
	Average	0·4137 ± 0·051	0·0026	1·512

Table 4. Comparisons of fitness (*W*) and relative fitness (*m*) of *D.s. neonasuta* as measured by the competition with *D.n. nasuta*, *D.n. albomicana* and *D. hypocausta*.

Species	m ± SE	W	P
D.s. neonasuta/D.n. nasuta *D.s. neonasuta/D.n. albomicana*	−0·1567 ± 0·058	0·8553	< 0·01
D.s. neonasuta/D.n. nasuta *D.s. neonasuta/D. hypocausta*	−0·4529 ± 0·058	0·6357	< 0·01
D.s. neonasuta/D.n. albomicana *D.s. neonasuta/D. hypocausta*	−0·2962 ± 0·058	0·7437	< 0·01

Thus, the differential ability of the species to exploit the experimental environment is striking. The comparisons of the parameters in the experimental populations can be summarized as follows: for productivity—*D.n. nasuta* > *D. hypocausta* > *D.s. neonasuta* = *D. pulaua* = *D.n. albomicana* = *D.s. bilimbata*; and for population size–*D.n. nasuta* > *D.s. neonasuta* > *D. hypocausta* > *D. pulaua* = *D.n. albomicana* = *D.s. bilimbata*. Essentially, the ability of a population to increase in numbers depends upon the birth rate and the survival rate of the animals. Those populations which have greater productivity have larger population size in the present experimental populations with the exception of *D. hypocausta*. Similar correlation between productivity and population size has been reported by Mourao *et al* (1972) in *D. willistoni* and Nirmala (1973) in *D. ananassae* and *D. melanogaster*.

Relevant literature reveals that there exist evidences both for correlation and lack of it, of the species performances in pure and mixed cultures. Tantawy and El-Wakil (1970) studied competition between *D. funebris* and *D. virilis* as well as fitness of these two species in isolation. On various fitness parameters, *D. funebris* was superior to *D. virilis* in isolation. However, in competition experiments, *D. funebris* was rapidly

Table 5. Mean values along with the standard errors of the productivity and population size in the population of 6 species.

Population	Species	Productivity	Population size
25	*D.n. nasuta*	70·00 ± 12·78	165·87 ± 16·35
26	„	71·20 ± 12·17	177·58 ± 18·93
27	„	67·91 ± 12·92	163·70 ± 21·66
28	„	85·75 ± 15·70	199·37 ± 24·04
	Average	73·71	176·63
29	*D.s. neonasuta*	52·54 ± 14·04	129·87 ± 16·33
30	„	46·20 ± 9·61	120·25 ± 18·08
31	„	42·00 ± 10·92	107·20 ± 17·11
32	„	40·54 ± 9·77	105·54 ± 14·81
	Average	45·32	116·71
33	*D.n. albomicana*	36·70 ± 7·85	74·04 ± 8·81
34	„	36·00 ± 7·91	68·79 ± 10·22
35	„	27·12 ± 5·35	56·95 ± 6·73
36	„	34·00 ± 7·76	70·20 ± 9·04
	Average	33·45	67·45
37	*D. pulaua*	45·25 ± 9·49	74·66 ± 12·13
38	„	34·95 ± 6·91	65·35 ± 8·88
39	„	32·83 ± 7·12	62·58 ± 8·15
40	„	37·79 ± 6·29	68·45 ± 8·43
	Average	35·20	67·76
41	*D.s. bilimbata*	32·75 ± 8·01	61·66 ± 7·75
42	„	31·66 ± 7·30	60·00 ± 8·32
43	„	36·54 ± 7·45	62·33 ± 8·52
44	„	35·33 ± 8·72	64·37 ± 8·93
	Average	34·07	62·09
45	*D. hypocausta*	32·71 ± 4·84	64·28 ± 6·06
46	„	56·14 ± 8·87	97·00 ± 12·88
47	„	68·57 ± 9·94	102·28 ± 13·87
48	„	72·57 ± 10·26	111·43 ± 16·27
	Average	57·49	93·74

eliminated. Barker and Podger (1970) found that *D. melanogaster* raised in mixed cultures were less fecund than those of pure cultures, while *D. simulans* showed reverse effect, in contrast to the reports of Ayala (1968, 1969b, 1970) and Nirmala (1973). They have demonstrated the existence of correspondence between adaptedness obtained from single species populations and those based on interspecies phenomena.

In the present experiments, *D.n. nasuta* out vies all the 4 species competitors namely, *D.s. neonasuta*, *D. hypocausta*, *D. pulaua* and *D.s. bilimbata*. These species are inferior to *D.n. nasuta* and hence there is a parallelism in the performances of these species in pure and mixed cultures. The relative fitnesses achieved by *D.n. nasuta* against these species differ significantly since it is an outcome of the competitive interaction of the two contesting species. Because of the least adaptedness of *D. pulaua*, *D.n. nasuta* was able to evince the highest fitness against it than against *D.s. neonasuta*, which has a better adaptedness than that of *D. pulaua*. *D.s. neonasuta* is a loser against *D.n. nasuta*, probably due to its lower adaptedness. Further, as *D.s. neonasuta* is superior to *D.n. albomicana* and *D. hypocausta*, it was able to supplant them in competition.

Thus, there exist evidences both for the negative and positive correlations between inter- and intra-specific competitive fitnesses of species. Competitive ability is the sum total of numerous factors that interact exceedingly in complex ways. Therefore, under these circumstances it is difficult to predict the outcome of interspecies competition by judging the species performances in pure cultures. The single species (pure cultures) and two species (mixed cultures) populations represent models of two levels of complexity. Therefore, these laboratory populations can be utilized as biological models to study the dynamics and the process of competition.

These taxonomically closely related species of *Drosophila* under experimentation have reacted differently to the present experimental set up, each manifesting different levels of adaptedness and relative fitness. Thus, the present findings suggest the lack of correlation between taxonomic nearness and the ecological phenotype of *Drosophila* species under investigation.

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Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera)

A C BHARDWAJ

PG Department of Zoology, Sanatan Dharm College, Muzaffarnagar 251 001, India

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Abstract. Enzyme system pertaining to the midgut region of *Adoretus lasiopygus* Burm. is studied in vitro. Most of the carbohydrases operate optimally in the mesomesenteron whereas proteinases and lipases behave optimally in the metamesenteron region. Optimum conditions of certain carbohydrases in relation to pH, temperature, substrate concentration and enzyme concentration have been studied. α-amylase, α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase showed optimum activity at pH 5·7, 6·2, 5·0, 5·8 and 5·6 respectively. α-Amylase, α-glucosidase and α-galactosidase are physiologically adapted to high temperature ranging between 48°–62°C. A positive response of starvation on the secretion of cellulase, inulinase, α- and β-galactosidase is observed. Based on this parameter the midgut enzymes of the pest is divided into two groups i.e. enzymes secreted continually and those secreted under the influence of food.

Keywords. Enzymes level; midgut; *Adoretus lasiopygus*; Scarabaeidae.

1. Introduction

Utilization of complex molecules in their food by animals is not possible unless the former get cleaved into simpler forms absorbable through the gut epithelium. The midgut region of several beetles is adapted physiologically to degrade complex molecules in their diet with the aid of appropriate enzymes secreted from the midgut epithelium. Krishna (1958), Bhatnagar (1962), Rao and Rastogi (1967), Sakurai (1968), Chinnery (1971), Baker (1976), Shukla and Upadhaya (1978) are some of the recent workers who have described enzyme systems pertaining to the digestion of food in various coleopteran families. The scarabaeid beetles are of great economic value but remained untouched for such study after Swingle (1930). The present communication describes exo- and endogeneous digestive enzymes of the Chafer beetle, *Adoretus lasiopygus* which feeds on the leaf lamina of its host plant, *Magnifera indica* Linnaeus.

2. Material and methods

The beetles used in the present investigation were collected from its host plant in the night during rainy season when they congregate in large numbers. Both field collected and 24 hours starved individuals were vivisected under stereoscopic binocular microscope in cold double distilled water. The midgut region freed from all adhering tissues was incised into morphological distinct parts as described earlier (Bhardwaj 1985). For enzyme assay extract of each part of midgut was prepared and stored under refrigerated conditions. The reaction and control mixtures were prepared (Goel and Bhardwaj 1981) and incubated at 37°C ± 2°C for about 5–10 hr under a layer of toluene. The incubated mixtures were analysed for the presence of hydrolytic product of carbohydrates (Plummer 1971), peptone, casein, lipid, ester (Baldwin and Bell 1967) and urea (Feigl 1947) as detailed elsewhere (Goel and Bhardwaj 1981).

To study the optimum pH of different carbohydrases buffers used were HCl-sodium citrate, 0·1 M for pH 3·5; Soresen's phosphate, 0·1 M for pH 5·5–8·0; glycine-NaOH, 0·1 M for pH 8·5–9·0. To obtain temperature optima of carbohydrases reaction mixtures were incubated at temperature ranging from 10°–70°C with an interval of 10°C. To study influence of enzyme concentration on the activity of carbohydrases extract of pro-, meso- and metamesenteron was prepared separately using 1 midgut region/ml to 6 midguts region/ml (w/v). Substrate concentration ranging from 1·0–8·0% was used to see its effect on the enzymes. The following colorimetric procedure was used for different parameters.

Appropriate buffer and substrate in equal proportions were mixed and incubated at 37°C for 10 min. Later 100 μl midgut extract was added and reincubated exactly for 60 min. The carbohydrase activity was terminated by adding 4 ml 3, 5 dinitrosalicylic acid reagent (Noelting and Bernfeld 1948; Plummer 1971). The mixture so obtained was heated for about 5 min at 100°C in water bath and followed by immediate cooling. The extinction was read at 540 nm with the help of colorimeter. A control and blank was also prepared and treated in the same way. Enzyme activity was expressed as difference in absorbance between reaction and control mixtures. The process was repeated 4 times and mean is taken.

3. Results and discussion

In vitro studies indicate that midgut of *Adoretus lasiopygus* is physiologically adapted to utilize starch, maltose, sucrose, raffinose, melibiose, cellobiose and lactose as main carbohydrates; simple proteins and very little fat occurring in the diet of pest. The relative strength of various enzymes is shown in table 1.

Starch constitutes major part of the diet in phytophagous beetles. Its quick hydrolysis is observed with pro- and mesomesenteron extract while reaction remained relatively slow with the extract of metamesenteron. As a result of starch hydrolysis the turbidity in the reaction mixture disappears. It indicates presence of dextrogenic or liquefying amylase (Fruton and Simmonds 1965). The analysis of the reaction mixture elucidates oligosaccharides on the chromatoplate thus confirms amylase activity. The amylase has cleaved glycosidic linkage of starch probably in the interior of the chain hence it is called as α-amylase in *A. lasiopygus* and supports Gilmour's (1962) view about the universal occurrence of α-amylase in insects. Applebaum (1964) classified amylase into α- and β-amylase while studying action pattern in the midgut of *Tenebrio* sp. larva. The nonspecific amylase has been reported earlier by several workers in the beetle they studied (Lal and Ghai 1958; Krishna 1958; Bhatnagar 1962; Krishna and Saxena 1962; Rao and Rastogi 1967; Sakurai 1968; Chinnery 1971; Shukla and Upadhaya 1978).

The digestion of inulin with metamesenteron extract is evident from the presence of fructose on the chromatoplate. The metamesenteron is the only region in the whole midgut of *A. lasiopygus* adapted to secrete inulinase and utilizes inulin before being passed out.

Among structural polysaccharides like cellulose, pectin and chitin; the cellulose could only respond to the metamesenteron extract. It suggests presence of cellulase in the said

Table 1. A summarised presentation of information relating to qualitative assay of digestive enzymes in the different regions of the midgut of *Adoretus lasiopygus* Burm.

E.C. number	Enzyme	Substrate	Enzyme distribution					
			Promesenteron		Mesomesenteron		Metamesenteron	
			Fed	Starved	Fed	Starved	Fed	Starved
	Carbohydrases							
3·2·1·1	α-Amylase	2% Starch	+ + +	+	+ + +	+	+ +	+
3·2·1·4	Cellulase	2% Carboxymethylcellulose	–	–	–	–	+	–
3·2·1·7	Inulinase	2% Inulin suspension (Aq.)	–	–	–	–	±	–
3·2·1·14	Chitinase	2% Chitin suspension (Aq.)	–	–	–	–	–	–
3·2·1·15	Polygalacturonase	Pectin suspension (Aq.)	–	–	–	–	–	–
3·2·1·20	α-Glucosidase	5% Maltose, sucrose	+ +	+ +	+ + +	+ +	+	+
3·2·1·21	β-Glucosidase	5% Cellobiose	+	+	+ +	+	+	+
3·2·1·22	α-Galactosidase	5% Raffinose, melibiose	–	–	+ +	–	–	–
3·2·1·23	β-Galactosidase	5% Lactose	+	–	+ + +	–	±	±
3·2·1·26	β-Fructosidase	5% Raffinose, Phenyl-β-fructoside	–	–	–	–	–	–
3·2·1·28	α, α-Trehalase	5% Trehalose	–	–	–	–	–	–
	Proteinases							
3·4·21·3	Protease-A	2% Casein	+	–	±	–	+ +	±
	Polypeptidase	2% Peptone	±	–	+ +	–	+ + +	±
	Lipases							
3·1·1·1	Carboxylesterase	Ethyl butyrate emulsion	–	–	+	–	+ +	–
3·1·1·3	Triacylglycerolipase	Olive oil emulsion	–	–	±	–	±	–
	Amidase							
3·5·1·5	Urease	1% Urea	–	–	–	–	–	–

+ + +, Brisk activity; + +, moderate activity; +, feeble activity; ±, trace activity, –, no activity.

region of *A. lasiopygus* and is a point of significance because hydrolysis of cellulose in the insects that survive on woody or fibrous diet is brought about by flagellates or bacterial flora of their gut (Cleveland *et al* 1934). Midgut and the caecal origin of cellulase in *Blaberus discoidalis* has been demonstrated by Ehrhardt and Voss (1962). Similarly cellulase has also been reported from the crop and midgut of *Byrsotria fumigata* (Fisk and Rao 1964) and in the salivary gland of cockroach (Wharton *et al* 1965). The pectin and chitin remained unaltered in the reaction mixture even after an increase in incubation time is given.

The occurrence of glucose and fructose on the chromatoplate as end products of the substrates maltose and sucrose confirmed the activity of α-glucosidase in *A. lasiopygus*. The activity is moderate in the promesenteron, accelerated in the mesomesenteron whereas it has dropped towards metamesenteron. In the absence of β-fructosidase activity in *A. lasiopygus* it is presumed that sucrose with α- and β-linkage is cleaved only by α-glucosidase. The presence of non specific glucosidase is an adaptation on the digestive part of *A. lasiopygus* to utilize more carbohydrates of the diet. The availability of α-glucosidase in the midgut of *Dermestes maculatus* and *Dactylosternum hydrophilioides* has been reported by Chinnery (1971) and Shukla and Upadhaya (1978) respectively and support present observation. Similar to the midgut of *Latheticus oryzae* (Bhatnagar 1962) and *Tribolium castaneum* (Krishna and Saxena 1962) *A. lasiopygus* also secretes β-glucosidase. In present instance β-glucosidase activity is observed moderate in the mesomesenteron whereas the activity droped on either side. The α- galactosidase of *A. lasiopygus* digests trisaccharides such as melibiose and raffinose specifically in the mesomesenteron and supports observation made by Krishna and Saxena (1962). The β-galactosidase established in *L. oryzae* (Bhatnagar 1962) and *T. castaneum* (Krishna and Saxena 1962) throughout the length of midgut, is observed with optimum activity only in the mesomesenteron of *A. lasiopygus*. Unlike Bhatnagar (1962), Sakurai (1968), and Chinnery (1971) trehalase activity could not be traced in any region of the midgut of present scarabaeid.

The midgut of *A. lasiopygus* is also able to utilize simple proteins present in the food. Among these peptone seems to be quickly degraded by polypeptidase in the mesomesenteron. The digestion of peptone however, becomes vigorous towards metamesenteron probably due to the substantial amount of appropriate enzyme and favourable medium (Bhardwaj 1982). The casein shows feeble digestion with the extract of metamesenteron perhaps due to the inadequate amount of protease—A. The present finding thus supports occurrence of slight proteolytic activity in the midgut of *Tenebrio molitor* and *T. castaneum* (Dadd 1956; Brik *et al* 1962). The end products of these proteins are further assayed either by secretion of aminopeptidase or carboxypeptidase as suggested by Wigglesworth (1965). Carboxylesterase shows feeble activity in mesomesenteron while the activity enhances to moderate in the metamesenteron region. Triacylglycerolipase shows activity in traces in both meso- and metamesenteron regions of *A. lasiopygus*.

The digestive enzymes level vividly reveal that the present pest has a complex modified midgut, demarcated physiologically into pro-, meso- and meta mesenteron. Each region shows optimum hydrolysis affinity towards particular types of substrate. Thus promesenteron digests starch, maltose and sucrose. Mesomesenteron is the chief site where hydrolysis of several carbohydrates and peptone takes place. The metamesenteron is adapted for the digestion of proteins and lipids. Such a specific digestive adaptation in *A. lasiopygus* is highly correlated with the nature and arrangement of epithelial cells in different regions as established histocytologically earlier (Bhardwaj 1985).

pH values for optimum activity of insect carbohydrases vary widely and range from 5·4–7·5 (Shinoda 1930; Rao and Rastogi 1967; Krishna 1958). The α-amylase activity of *A. lasiopygus* is quite appreciable in a pH zone 3·5–7·0. The optimum amylolytic activity is recorded at pH 5.7 (figure1) which is almost similar to the optimum amylase pH of *Epilachna vigintioctopunctata* (Sahurai 1968). Krishna and Saxena (1962) established slight low pH optimum for the activity of amylase in *T. castaneum* larva and adult. The α-glucosidase behaves appreciably between pH zone 4·7–6·8. Its optimum activity takes place at pH 6·2 (figure 2). The β-glucosidase appears to be capable of operating under pH ranging from 3·8–6·3. Its optimum activity is noticed at pH 5·0 (figure 3). The α-galactosidase has an optimum pH 5·8, although this enzyme has noticeable catalytic activity between pH 5·5–6·5 (figure 4). Krishna and Saxena (1962) recorded pH optimum 5·4 for this enzyme. The β-galactosidase activity falls into relatively wider pH zone which ranges from 4·5–6·6. It has optimum activity at pH 5·6 (figure 5) an observation almost parallel to Krishna and Saxena (1962).

There is progressive response in the activity of carbohydrases of *A. lasiopygus* with the rise of temperature. It has been observed that α-amylase, α-glucosidase and α-galactosidase behave optimally at 55°C. Higher temperature stimulates amylolytic activity in *T. molitor* (Jankovic *et al* 1976). In the present case further rise in temperature drops enzymatic activity sharply and ceases almost at 68°C. This suggests that carbohydrases of *A. lasiopygus* are physiologically adapted to operate at moderately

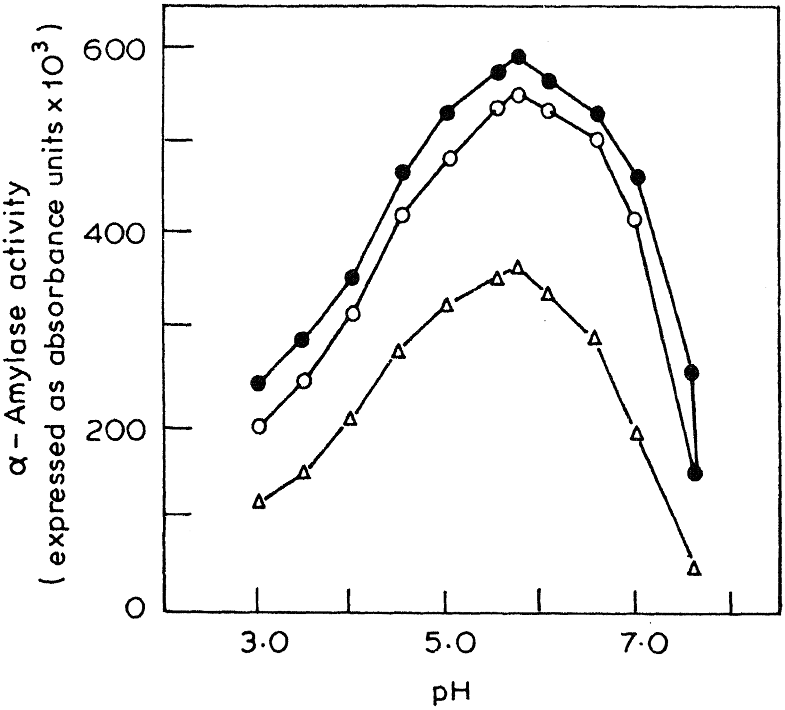

Figure 1. pH profile of α-amylase in midgut regions of *A. lasiopygus*. ●——● Promesenteron, ○——○ Mesomesenteron, △——△ Metamesenteron.

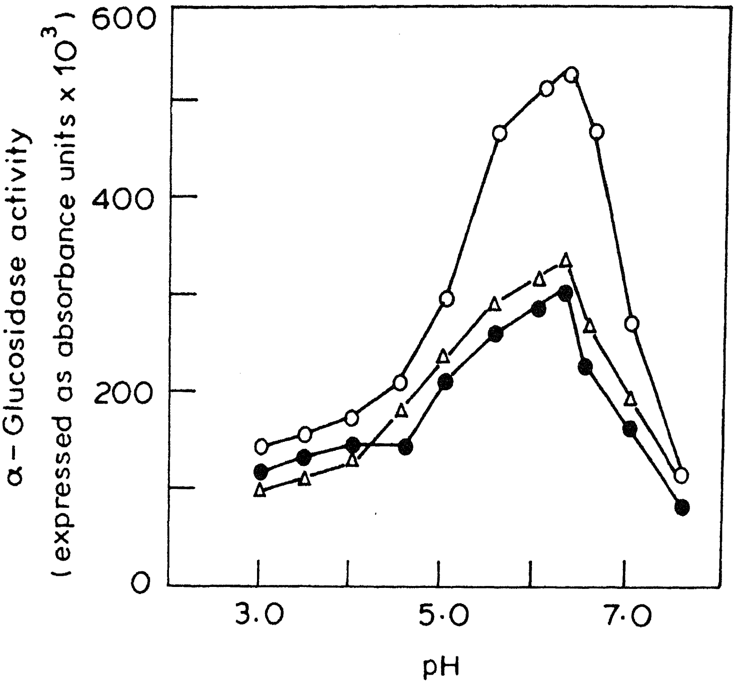

Figure 2. pH profile of α-glucosidase in midgut regions of *A. lasiopygus*.

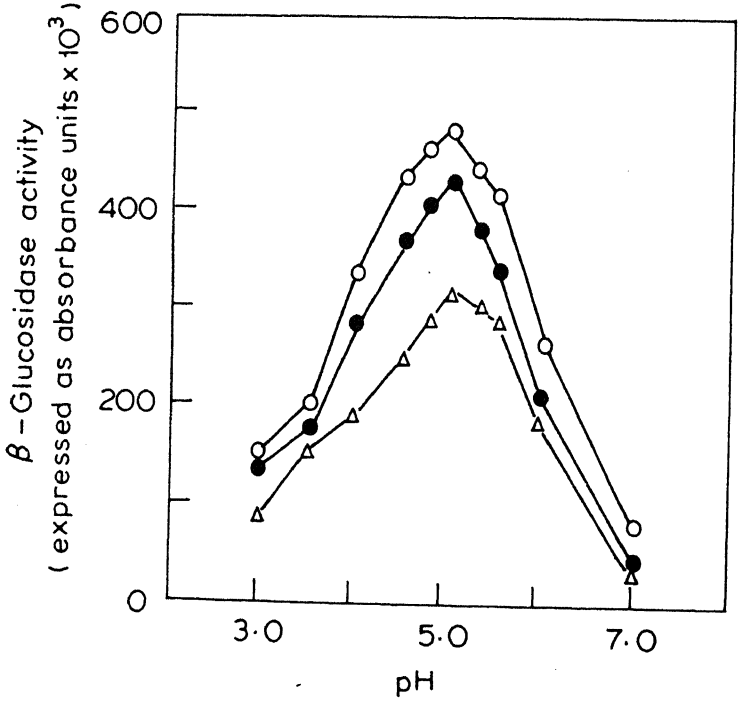

Figure 3. pH profile of β-glucosidase in midgut regions of *A. lasiopygus*.

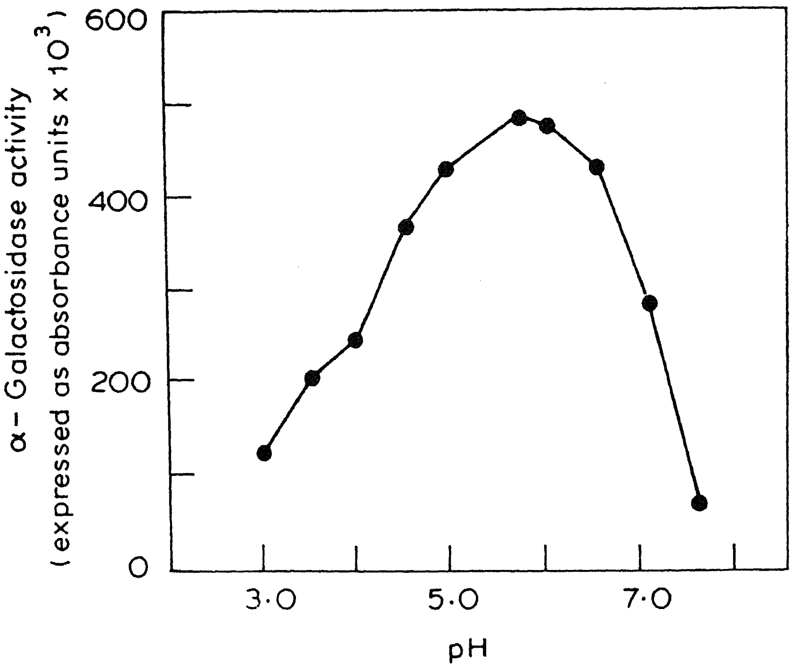

Figure 4. pH profile of α-galactosidase in the mesomesenteron of *A. lasiopygus*.

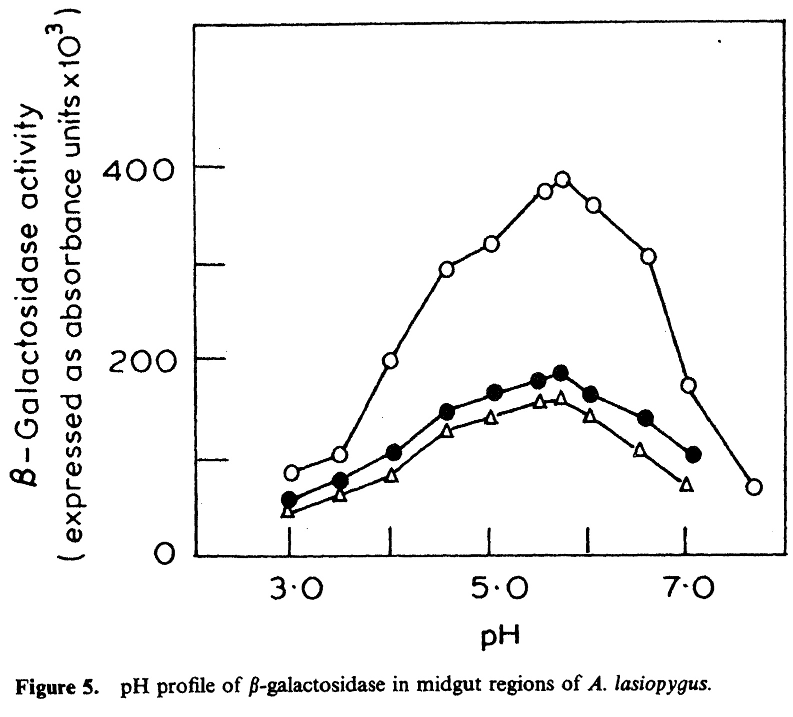

Figure 5. pH profile of β-galactosidase in midgut regions of *A. lasiopygus*.

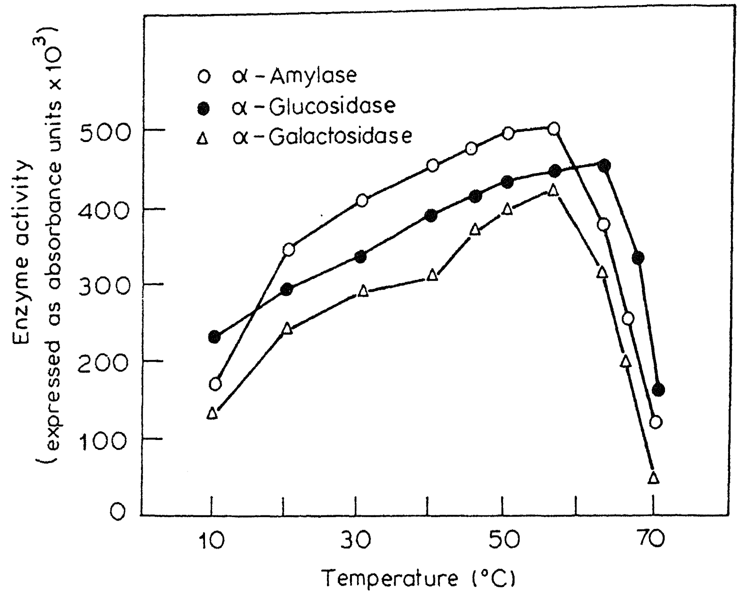

Figure 6. Impact of temperature on the activity of certain midgut carbohydrases of *A. lasiopygus*.

high temperatures and behave appreciably at normal temperatures i.e. from 30°–37°C at which this pest flourishes (figure 6). There is a significant variation in the optimum temperature for amylase activity. It ranges from 28°–60°C (Day and Powning 1949; Applebaum *et al* 1961; Rao and Rastogi 1967; Sinha 1975; Jankovic *et al* 1976; Hori 1970) in different groups of insects.

Five substrates with increasing concentration ranging from 1–8 % were used to assess the influence of it on the activity of respective enzyme. The results indicate that amylolytic activity is quite moderate even at 1 % concentration of starch is used. There is very little augmentation in enzymatic activity with further increase in concentration. The α-galactosidase shows feeble rise in activity with rise of raffinose concentration, beyond 4 % the reaction becomes more or less constant. Likewise 4 % concentration of lactose seems to be appropriate for β-galactosidase activity. The α-glucosidase activity enhances sharply with the rise of cellobiose concentration upto 5 %. Further addition of substrate shows a decline in enzymatic activity whereas the activity of β-glucosidase shoots up with increasing concentration of maltose.

Amylase activity in *A. lasiopygus* shows a linear progressive response with the rise of enzyme concentration up to a certain limit (5 midguts/ml). Further rise in enzyme concentration shows feeble amylolytic activity thereafter hydrolysis of starch becomes constant. A much higher enzyme concentration is recorded for the hydrolysis of starch (6–12 midguts/ml) in *Sarcophaga ruficornis* and *Musca domestica* (Sinha 1975).

A significant positive effect of starvation on the secretion of cellulase, inulinase, α-, β-galactosidase, proteinases and esterases have been observed. The remaining carbohydrases are secreted continually into the midgut lumen but their secretion however, enhance as food comes in the midgut. Probably the presence of some food molecules stimulate midgut epithelium for secretion. It is also interesting to note that a particular enzyme in the region of promesenteron inhibits under starvation but this parameter fails to arrest the secretion of same enzyme in the succeding regions or *vice versa* (table 1). It is believed here that different enzymes are secreted from different epithelial cells of the same region. A particular sector among these cells responded to the starvation while remainder cells behaved normally. Thus based on this parameter the enzyme system of *A. lasiopygus* can be divided into two groups i.e. enzyme secreted continually and those secreted under the influence of food.

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Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid fish, *Oreochromis mossambicus* (Peters), with special reference to swimming speed

N SUKUMARAN*

Fisheries College, Tamil Nadu Agricultural University, Tuticorin 628 008, India

*Present address: Agricultural Research Station, Tamil Nadu Agricultural University, Bhavanisagar 638 451, India

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Abstract. Oxygen consumption, carbon dioxide output, nitrogen excretion, respiratory quotient, ammonia quotient and nitrogen quotient in cichlid fish, *Oreochromis mossambicus* (Peters) acclimated to and tested in freshwater at 30°C, were obtained with special reference to swimming speed (20–77 cm/sec). Tilapia continuously swimming for 5 hr derived energy both from aerobic and anaerobic sources during the initial phase of exercise, whereas during the later phase the energy utilization is completely from aerobic source, as it is evident from respiratory quotient values. It also utilized more proteins for energy during the later phase of exercise, as evident from the increased ammonia-N as well as total N excretion and the respective quotients. The increased protein utilization may be of advantage in maintaining acid-base balance and also in conserving (Na^+) in fish.

Keywords. Oxygen consumption; nitrogen excretion; RQ, AQ and NQ.

1. Introduction

Considerable information has been accumulated on metabolism of fish subjected to exercise (Spoor 1946; Wohlschlag 1957; Beamish 1970; Smit 1965; Smit *et al* 1971; Webb 1971). Although anaerobic metabolism in fish forced to swim has not been investigated in the previous studies, Kutty (1968) and Sukumaran and Kutty (1977) studied the changes of respiratory quotient (RQ) in relation to swimming speed and duration of exercise in gold fish and freshwater mullet, respectively. Sukumaran and Kutty (1979) studied the RQ and nitrogen excretion in *Rhinomugil corsula* exercised for hours together and observed that while anaerobic energy utilization was high during the initial phase of exercise, protein utilization increased with the increase in the duration of exercise. The objective of present study is to find out the extent of anaerobic metabolism in the Cichlid fish, *Oreochromis mossambicus* subjected to continuous exercise for 5 hr at various swimming speeds and to find out the quantum of protein utilization during long-term swimming as judged from ammonia quotient (AQ) and nitrogen quotient (NQ).

2. Materials and methods

Tilapia (ranging from 16·7–19·3 cm in length and 33·9–53·1 g in weight) were collected from the freshwater tanks in and around Madurai and held in holding tanks in the laboratory. They were acclimated to freshwater at 30 ± 1°C atleast two weeks before the experiment and tested under acclimation conditions. During acclimation they were fed *ad lib* with a formulated food: fish muscle, goat liver and wheat hearts in 2:1:1 ratio (Sukumaran and Kutty 1977). The fish were starved for 24 hr (Beamish 1964) before experiment.

The apparatus used for the present study is an adaptation of Blazka's respirometer of 7·5 litres capacity (Blazka *et al* 1960; Smit 1965; Sukumaran and Kutty 1977). This is made up of two concentric cylindrical tubes of transparent acrylic plastic. One end of outer tube is fixed to a propeller attached to an electric motor (0·25 HP). By regulating the speed of the motor with a step-down transformer, water inside the swimming chamber is recirculated at different current speeds. The apparatus was partially covered by a black plastic curtain to safeguard the fish from external disturbances.

Four different swimming speeds were chosen for the present study namely 20, 38, 61 and 77 cm/sec. The fish was introduced into the swimming chamber 12 hr prior to experiment in order to avoid handling effect on metabolism (Brett 1964). The rest fish was allowed to swim at one particular swimming speed continuously for 5 hr and samples were taken for analysis at the beginning and end of 1st hr, 3rd hr and 5th hr with 1 hr closure of the apparatus in each case. During the remaining hours of exercise the apparatus was flushed with well aerated water under acclimation conditions. This study was restricted to only at 30°C.

The unmodified winkler method was followed (American Public Health Association 1965) for analysing dissolved oxygen in water. Total carbon dioxide was measured by Maros-Schulek technique modified for fish metabolic studies by Kutty *et al* (1971). Ammonia was measured spectrophotometrically using the phenol-hypochlorite method with ammonium chloride standards (Solorzano 1969).

Total N in water was measured by micro Kjeldahl method. 25 ml of the sample was digested with digestion mixture and 5 ml of concentrated sulphuric acid and subsequently determined the total N using micro Kjeldahl apparatus.

3. Results

The metabolic rates, oxygen consumption, carbon dioxide output, the corresponding RQ, Ammonia excretion, the corresponding AQ, total N excretion and the corresponding NQ were measured in Tilapia (*O. mossambicus*) forced to swim continuously for 5 hr at each swimming speed (20, 38, 61 and 77 cm/sec) at 30°C. The data on oxygen consumption, carbon dioxide output and the corresponding RQ are plotted against swimming speed of tilapia in figure 1. The plots of NH_3-N excretion and total N excretion in semilogarithmic grid against swimming speed are presented in figure 2. The data on AQ, NQ and the percentage of non-protein N in total nitrogen excreted against swimming exercise are plotted in figure 3.

The individual determination obtained for 1st, 3rd and 5th hr exercise are presented by separate symbols in each case. Regression lines have been fitted for the semi-logarithmic plots separately for each hour of exercise and respective regression equations including standard errors for '*a*' (y intercept) and '*b*' (slopes) are given in table 1.

From the plots of figure 1, it is evident that the rates of oxygen consumption and carbon dioxide output increase with increase in swimming speed and decrease with duration of exercise. The plots of RQ indicate positive slope with increase in the intensity

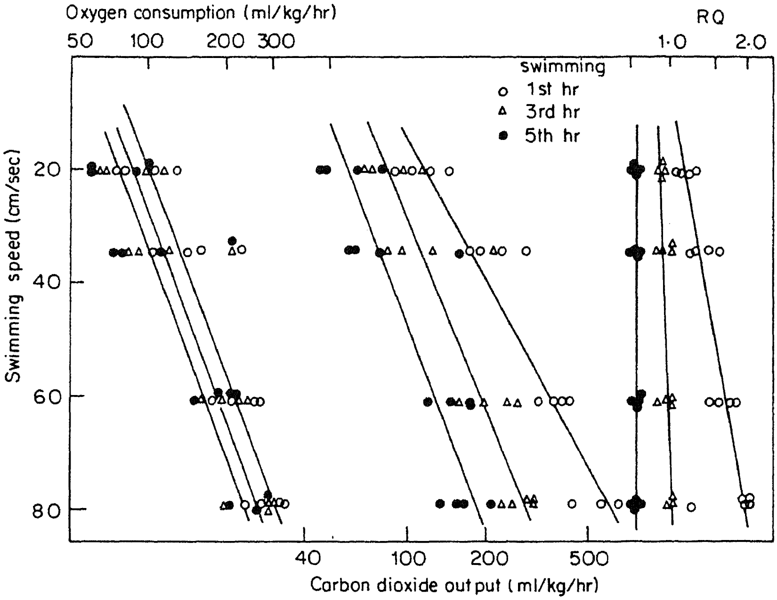

Figure 1. Oxygen consumption, carbon dioxide output and RQ in *O. mossambicus* acclimated and tested at 30°C in freshwater plotted against swimming speed. The lines are fitted as per the regression equations given in table 1.

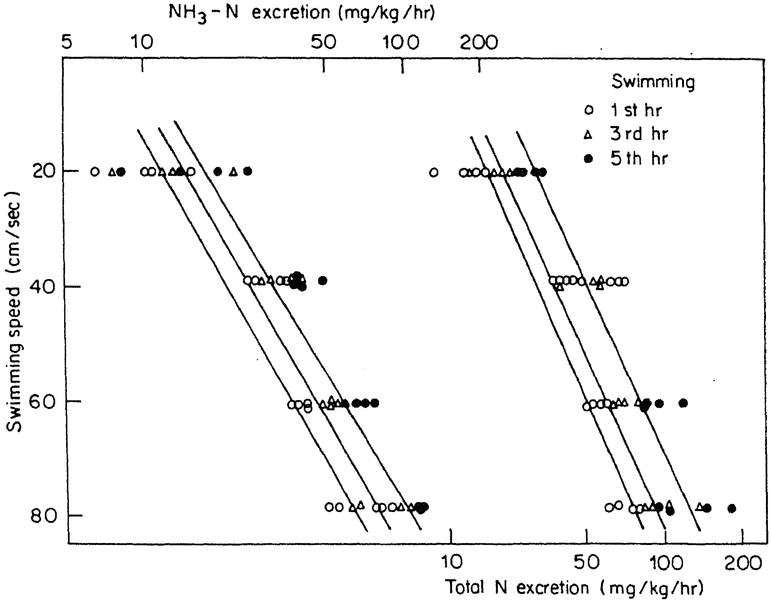

Figure 2. Ammonia-N excretion and Total N excretion in *O. mossambicus* acclimated and test at 30°C in freshwater plotted against swimming speed. For other details, see figure 1.

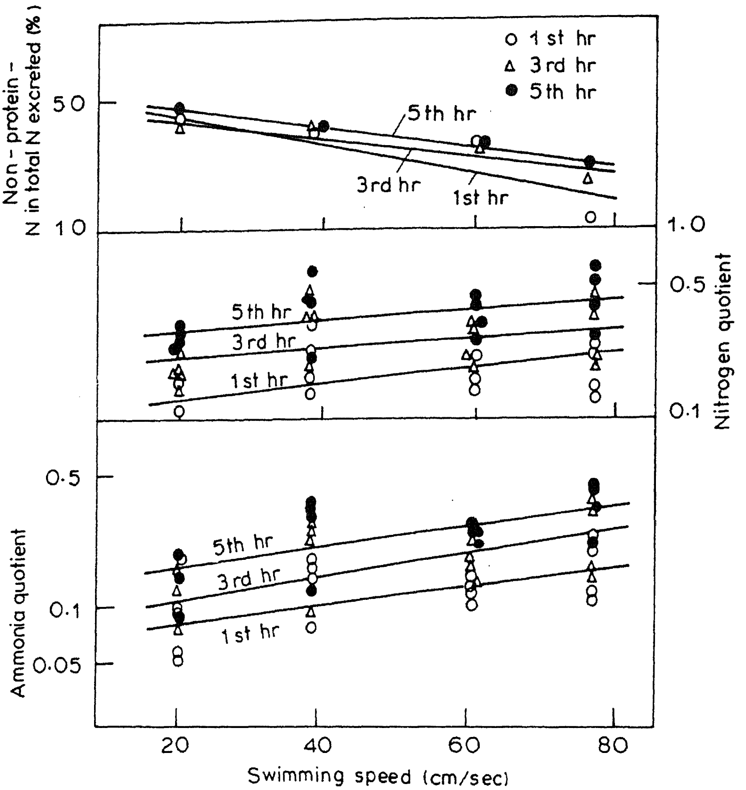

Figure 3. AQ, NQ and percentage of non-protein-N in total N excreted by *O. mossambica* acclimated and tested at 30°C in freshwater plotted against swimming speed.

of exercise during 1st hr of swimming, followed by a negligible slope with increase in exercise during the 5th hour of swimming. It is evident from figure 2 that the rates of ammonia excretion and total N excretion increase with the increase of swimming speed indicating the higher utilization of protein during strenuous exercise. The plots of AQ and NQ (figure 3) show the same trend of increase with increase in the intensity and duration of exercise. The percentage of non-protein nitrogen in total N excreted, decreases with the increase of exercise (figure 3).

4. Discussions

As observed earlier in other species (Kutty 1968; Sukumaran 1976; Sukumaran and Kutty 1977) in *O. mossambicus* also, the oxygen consumption and carbon dioxide output increase with increase of swimming speed and decrease with the duration of exercise (figure 1). The slope ('*b*' values) of regressions on oxygen consumption and carbon dioxide output with swimming speed for Tilapia are greater than those observed for *R. corsula* (Sukumaran 1976), indicating that increase of metabolism with swimming effort is much higher in *O. mossambicus*. Such steep curve of oxygen consumption has been observed in sockeye salmon (Brett 1964), gold fish (Smit 1965;

Table 1. Regression equations of logarithms of O_2 consumption, carbon dioxide out (ml/kg/hr), NH_3-N excretion, total N excretion, RQ, AQ, NQ (Y in each case) in *O. mossambicus* against swimming speed (cm/sec). Data obtained for 1st, 3rd and 5th hr of 5 hr continuous exercise at 30°C.

	Exercise (hr)	Regression equation $\log Y = a + bX$
		Log $Y =$
Oxygen consumption	I	1·849228 (±0·039644) +0·0081025 (±0·0004656)X
	III	1·776708 (±0·0654606)+0·008199 (±0·0006035)X
	V	1·744077 (±0·0686438)+0·007985 (±0·0009746)X
Carbon dioxide output	I	1·829238 (±0·0289063)+0·120696 (±0·0003050)X
	III	1·750921 (±0·0650767)+0·008876 (±0·0007159)X
	V	1·606985 (±0·0805283)+0·008266 (±0·0012464)X
RQ	I	−0·021944(±0·0120412)+0·003984 (±0·0002375) X
	III	−0·050417(±0·0116336)+0.000662 (±0·0003397)X
	V	−0·134931(±0·0174395)+0·000249 (±0·0001313)X
NH_3-N excretion	I	0·840310 (±0·0869680)+0·012946 (±0·0017537)X
	III	0·922147 (±0·1066909)+0·013203 (±0·0020357)X
	V	0·981945 (±0·1119206)+0·013850 (±0·0020562)X
Total N excretion	I	1·136713 (±0·0503319)+0·009988(±0·0001031)X
	III	1·215973 (±0·0393381)+0·010151(±0·0012389)X
	V	1·321158 (±0·036958) +0·010657(±0·0014742)X
AQ	I	−1·164583(±0·0512568)+0·004859(±0·0013762)X
	III	−1·083965(±0·1186054)+0·006618(±0·0020889)X
	V	−0·879249(±0·087217) +0·005274(±0·0017026)X
NQ	I	−0·874055(±0·0445493)+0·002878(±0·0003831)X
	III	−0·735303(±0·0528675)+0·002658(±0·0004731)X
	V	−0·577897(±0·0276903)+0·002546(±0·0006040)X

Values in parentheses indicate one standard error ($N = 16$ in each case).

Kutty 1968) and rainbow trout (Kutty 1968). The initial (1st hr) RQs of *O. mossambicus* are always higher than unity, whereas the steady (long term) RQs are below unity (figure 1) suggesting that anaerobic energy utilization is high during the initial phase of swimming exercise, but not during later phase as again observed in *R. corsula* (Sukumaran 1976). The initial increased anaerobic metabolism is possibly due to excitement (Brett 1964; Smit 1965; Kutty 1968; Smit *et al* 1971; Sukumaran and Kutty 1979). However, it should be noted that oxygen uptake during the 1st hr of swimming is also higher, so that both aerobic and anaerobic metabolism are initially higher possibly due to excitement, which apparently decreases with the increase in the duration of exercise. In sockeye salmon swimming at high speed, (Brett 1964) found that there is considerable amount of anaerobic energy utilization as judged from oxygen debt. Kutty (1968) found that during sustained swimming at 20°C in fully air saturated water, the mean RQ during the 1st hr of exercise amounted to 1·66 and during hours 3–12 it stabilised at about 0·95. The initial RQ of *O. mossambicus* is around 2·0 in sustained swimming, while the long-term RQ are below unity and values are close to protein fat value. Kutty (1968) and Sukumaran (1976) suggested more of protein or fat utilization in gold fish and *R. corsula* during long-term swimming at high speeds. Kutty's (1972) preliminary experiments on *O. mossambicus* forced to swim for 6 hr at a single speed confirmed that there is considerable involvement of protein metabolism during long-term swimming. Brett (1973) demonstrated in sockeye salmon, *Oncorhynchus nerka* that in the case of starving animal, fat and protein are the major fuel sources to provide energy for the initial phase of prolonged exercise, although metabolizable carbohydrate would be present in the liver.

Nitrogen (ammonia and total) excretion increases sharply with increase in duration of exercise and intensity of swimming speed (figure 2) indicating the involvement of more protein in energy budget during long-term swimming in *O. mossambicus* as observed in other fishes (Sukumaran and Kutty 1977, 1979). The respective quotients (AQ and NQ) also show the same trend indicating the utilization of more protein during the long-term swimming. In this context, it is worth mentioning that Jobling (1980) studied the AQ and muscle protein depletion during starvation of plaice, *Pleuroneatus platessa* L. and concluded that AQ may prove to be a useful measure of energy utilization in starving fish.

A salient point which comes out from the present study is that there is decrease in oxygen consumption and increase in AQ and NQ as observed in *R. corsula* (Kutty and Mohamed 1975) under hypoxic conditions and in *Mystus armatus* (Sukumaran and Kutty 1977) under forced activity. The rise in AQ and NQ indicates the relative increase in protein utilization. The NQ values of *O. mossambicus* during initial phase of exercise is well below the aerobic maximum of 0·148 based on mammalian energetics, but has been revised for fish system with the assumption that the nitrogenous end product is ammonia (Kutty 1975; Sukumaran 1976), while the NQ during long-term swimming shootup above 0·5 in high speed swimming. This rise in NQ clearly indicates the extra release of nitrogen anaerobically as observed in *R. corsula* (Sukumaran 1976) and in *M. armatus* (Sukumaran and Kutty 1977). Driedzic and Hochachka (1976) reported that amino acids were utilized as an anaerobic energy source in carp white muscle and also postulated that adenylate is the potential source of anaerobic ammonia.

NH_3-N forms about 65–70% of the total nitrogenous waste in *O. mossambicus* as observed in other fishes (Wood 1958; Fromm 1963; Forster and Goldstein 1969). In the present study a better picture is obtained by measuring the total N excretion. The percentage of NH_3-N in total nitrogen vary with the increase in duration of exercis well as the intensity of exercise (figure 3). These observations indicate that n ammonia fraction slightly increases with the duration of exercise in the high swimming speed tested as observed in *R. corsula* (Sukumaran 1976). Brett and Z (1975) studied the pattern of nitrogen excretion in sockeye salmon and found that urea nitrogen stays steady throughout the experiment, while the ammonia-nitro excretion shoots up after the fish was fed. The urea-N in the same species was found be 15 % of total N excretion and 70 % was accounted for NH_3-N. The remaining 1 of nitrogen was accounted for by amino and other nitrogenous waste like amino ac nucleotides etc. In the present study if the urea-N as seen in sockeye salmon, is assun to be constant, the minor change of non-ammonia fraction with the duration of exer in highest swimming speed may be caused by changes in the excretion of amino ac nucleotides etc.

It has been further suggested (Kutty 1972) after the observations of Maetz a Garcia Romeu (1964) and Garcea Romeu and Motais (1966), that NH_3 released can used to buffer the acid produced during anaerobic metabolism and in freshwater NH_4^+ ion is subsequently exchanged at the gills for sodium ions (Na^+). Thus increa NH_3 production in fish during stress conditions may be a mechanism for acid-b balance and also for conservation of Na^+. It is also suspected that at the beginning exercise the fish will lose some amount of Na^+ to outside from blood (Umminger a Gist 1972). Randall *et al* (1972) have also shown that active fish lose much more of N than resting fish. So it is likely that the increased excretion of ammonia during la phase of exercise may be to restore the Na^+, lost during the initial phase of exerc

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Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons

SHILLA CHATTERJEE, DHANANJAY PAL and ASOK GHOSH
Histophysiology Laboratory, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

MS received 17 August 1984; revised 30 March 1985

Abstract. Administration of vitamin C to juvenile castrated pigeons leads to a fall in the adrenal epinephrine level, but no significant change in the norepinephrine level is noted. Since the role of vitamin C in converting dopamine to norepinephrine is anticipated, it has been surmised that the bursa of Fabricius (thymolymphatic organ) might be responsible for the inability of these young birds to respond to vitamin C treatment.

Keywords. Vitamin C; adrenal medulla; epinephrine-norepinephrine content; thymolymphatic system; gonadectomy.

1. Introduction

The activity of the avian adrenal gland is considerably influenced by ascorbic acid or vitamin C (Ghosh and Chatterjee 1981). The adrenal glands contain the highest concentration of this vitamin. The latter appears to be one of the cofactors involved in the formation of norepinephrine (NE) from Dopamine (Levin *et al* 1960). The final step of biosynthesis, i.e. the methylation of NE to epinephrine (E) is dependent on the enzyme phenylethanolamine N methyl transferase (PNMT), which, in turn, is influenced by adrenal corticosteroids (Pohorecky and Wurtman 1971; Wasserman and Bernard 1971). Interestingly, it has been found that adrenal corticosteroid biosynthesis has been reported to be inhibited by vitamin C (Kitabchi 1967). Lower yield of corticosteroids would therefore retard the methylation of NE to E by PNMT.

Gonadal steroids are also alleged to affect the methylation mechanism of NE to E in birds (Sitaraman and Ghosh 1977; Chaudhuri *et al* 1966) and mammals (Luplescu 1958). Ljunggren (1969) has noted that in *Columba palumbus*, the degree of methylation of NE to E is perhaps greatest during the periods of lowest reproductive activity. Since these data are relatively old and based mostly on cytochemical observations, it seems essential to reinvestigate the problem by taking a more modern approach. As the first step in this direction, we have tried to investigate the effect of vitamin C on adrenomedullary catecholamines from a biochemical point of view. The experiment has been performed by eliminating gonadal influence in young adult pigeons, *Columba livia*.

2. Materials and methods

Thirty six young adult pigeons (about 2 months old) were procured from a local bird dealer and housed under conditions of the laboratory for a number of days. Young adult pigeons were chosen for convenience in surgical gonadectomy. Twelve birds were divided into two groups. While distilled water was administered intramuscularly to one group (Group I) at a dose of 0·8 c.c. water/bird/day for 7 days, the remaining birds (Group II) received intramuscular injections of vitamin C at a dose of 100 mg/100 gm body weight for 7 days. Of the remaining 24 birds, 6 were left intact and 18 birds were castrated. The wound was allowed to heal prior to the commencement of experimental therapies. The birds were divided into 3 equal groups (Groups IV, V and VI). While Group IV was left untreated, Groups V and VI received intramuscularly water and vitamin C, respectively, at a dose of 0·8 c.c. water/bird/day for 7 days and 100 mg/100 gm body weight for 7 days.

At the termination of the experimental period, the birds were killed by cervical dislocation, their adrenals were dissected out and either fixed in Wood's fixative for cytological procedures, or processed for biochemical quantitation of adrenomedullary catecholamines and adrenal ascorbic acid following von Euler's (1950) and Bessey *et al*'s (1947) methods, respectively.

3. Results

3.1 *Biochemical*

The biochemical data are summed up in table 1. Though not much change has been achieved in different groups, vitamin C therapy causes a fall in the epinephrine level of castrated young adult birds.

3.2 *Cytological*

The sections of adrenal glands fixed in Wood's fixative were observed under the light microscope after staining them with Wood's stain.

Castration leads to no change in the adrenomedullary picture. While slight preponderance of red (E) cells is seen in all control sections, predominance of yellow (NE) cells, and reduced staining intensity of red cells is seen in vitamin C treated sections. It is noteworthy that the cortex is atrophied in castrated pigeons, in contrast to previous reports of hypertrophied cortex in castrated ducks and fowls (Assenmacher 1973). Vitamin C treatment leads to further cortical atrophy.

4. Discussion

The present investigations reveal that castration does not affect adrenal catecholamine levels in young adult pigeons. Though adrenomedullary catecholamine levels do not change in ascorbic acid treated normal birds, in castrated birds, the administration of vitamin C causes a preferential release of epinephrine from the adrenal gland. The NE level, however, remains unchanged. The presence of vitamin C perhaps prevents the release of NE (cf. Subramaniam 1977). Secondly, there might be further retardation in the conversion of NE to E in gonadectomised birds, therefore indicating an inhibitory effect of vitamin C on PNMT activity. The retardation of PNMT activity by vitamin C seems to be more pronounced in the absence of gonads in young adult birds.

Table 1. Alteration in adrenomedullary hormones by vitamin C in intact and castrated young adult pigeons?

Group		Adrenal ascorbic acid (mg/100 gm tissue)	Epinephrine (μg/mg tissue)	Norepinephrine (μg/mg tissue)	Total catecholamine (μg/mg tissue)
I	Intact control (6)[a]	79·33 ± 21·53[c]	2·29 ± 0·19 (61 %)	1·45 ± 0·19 (39 %)	3·74 ± 0·22
II	Vitamin C (6)	68·71 ± 24·41	2·33 ± 0·21 (57 %)	1·78 ± 0·25 (43 %)	4·11 ± 0·28
III	Intact control (6)[b]	74·10 ± 5·30	1·36 ± 0·15 (56 %)	1·07 ± 0·23 (44 %)	2·43 ± 0·39
IV	Castrated control (6)[d]	63·80 ± 6·20	1·44 ± 0·14 (50 %)	1·44 ± 0·41 (50 %)	2·88 ± 0·55
V	Castrated Sham control (6)[d]	84·20 ± 15·40	1·46 ± 0·23 (57 %)	1·12 ± 0·16 (43 %)	2·58 ± 0·40
VI	Castrated + vitamin C (6)[d]	90·70 ± 1·60	0·66 ± 0·12 (34 %)[e]	1·28 ± 0·21 (66 %)	1·94 ± 0·32

Number in parentheses indicates number of specimen.

[a] Intact control for acute experiment with vitamin C.

[b] Intact control for other castrated groups, which had to be caged for a longer period.

[c] Mean ± Standard error.

[d] Castrated group.

[e] P value < 0·025.

Our previous experiments (unpublished results) with vitamin C administration to adult pigeons (where the bursa of fabricius normally atrophies) and bursectomised young pigeons led to rise in the norepinephrine level. This suggests that Dopamine β-hydroxylase, which occurs in the chromaffin granules of the adrenal medulla, accumulates ascorbate efficiently, and utilises it in the conversion of dopamine to norepinephrine. No significant rise in the NE level of young adult intact or castrated pigeons after vitamin C administration suggests that in these birds the homoeostatic mechanism concerning adenohypophysis-adrenal axis is somehow not yet perfected for effective functioning. The bursa of Fabricius (a thymolymphatic organ), which modifies many normal physiological responses of adult birds, is perhaps involved in the inability of young birds to respond to vitamin C treatment (compare Bhattacharya and Ghosh 1970). Probably many interesting facts might come out on repeating these experiments on adult and bursectomised young adult birds.

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Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål)*

KASI VISWANATHAN and M B KALODE
Directorate of Rice Research (AICRIP), Hyderabad 500030, India

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Abstract. Intensive host range studies comprising of 55 common weeds and eight cultivated plant species indicated that *Nephotettix virescens* could survive and breed only on susceptible rice variety, while *N. nigropictus* had a wider host range consisting of rice, sugarcane and five graminaceous weeds. *N. virescens* preferred rice plant both for settling and egg laying, while *N. nigropictus* showed greater preference to *Leersia hexandra* than others. Both species selected the host plants by gustation rather than by vision or olfaction.

Keywords. Host specificity; *N. virescens*; *N. nigropictus*; host selection; host range.

1. Introduction

In nature, the feeding privileges of phytophagous insects are restricted by biophysical and/or biochemical mechanisms. An understanding of the relationship between the host plant and insect can greatly contribute to develop pest management strategies.

The green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål) have become one of the serious pests of rice. Besides direct damage, both species can transmit important virus diseases. Thousands of hectares of rice were destroyed in 1971 and 1972 by tungro virus, transmitted by *N. virescens* in the Philippines (IRRI 1979).

N. virescens feeds on many grasses and weeds (Alam and Islam 1959; Pathak 1968; Misra and Israel 1970; Rao and Anjaneyulu 1977). Prasada Rao and John (1974) reported that in the absence of rice, *N. virescens* can thrive on weeds. Alam (1974) recorded *Leersia hexandra* as the principal host for *N. virescens*. However, many of these studies on host range did not provide sufficient supporting data. Therefore, detailed studies were conducted on the host specificity of *N. virescens* and *N. nigropictus* and results are presented in this paper.

2. Materials and methods

2.1 *Rearing and maintenance of insects*

Nephotettix virescens and *N. nigropictus* adults were collected from experimental plots of the All India Coordinated Rice Improvement Project. The species isolated and pure colonies were maintained in the greenhouse on potted plants of susceptible variety TN 1 in separate cages. Rearing cages were of 70 × 62 × 75 cm dimension and had glass panels on one side and the remaining sides were covered with fine nylon wiremesh.

2.2 *Host range*

Thirty two species of grasses, nine species of sedges, fourteen species of broad leaved weeds and eight cultivated plants were included for host range study (table 1). They were grown separately in pots and young and healthy plants were used for experimentation. Insect survival tests were conducted by releasing ten first instar nymphs and two pairs of adults per plant separately in suitable mylar film cages. Ten plants each were used as replications in both experiments. Observations on survival

Table 1. List of plant species tested in host range studies

Plant species
WEEDS
Cyperaceae
Cyperus compressus Linn.
C. difformis Linn.
C. distans Linn. f
C. rotundus Linn.
Fimbristylis miliacea Vahl
Fuirena pubescens Kunth.
Kyllinga triceps Rottb.
Scirpus erectus Poir.
Scirpus maritimus Linn.
Gramineae
Alloteropsis cimicina Stapf.
Aristida hystrix Linn. f
Bruchiaria ramosa (L.) Stapf.
B. reptans (L.) Gard. et C. L. Hubbard
B. mutica (Forssk) Stapf
Chloris barbata Sw.
C. virgata Sw.
Cymbopogon caesius Stapf.
Cynodon dactylon Pers.
Dactyloctenium aegyptam (L.) P. Beauw.
Dichanthium aristatum (Poir) C. E. Hubb.
Digitaria ciliaris Pers.
Denetra retroflex (Vaul) Pauzr.
Echinochloa colonum Link.
E. crusgalli Beauv.
Eleusine indica Gaertn.
Eragrostis pilosa Beauv.
E. gangetica Steud.
E. tenella R. & S.
Eragrostiella brachyphylla Staep.
Eriochloa procera Hubbard.
Imperata cylindrica Beauv.
Leersia hexandra Sw.
Oryza officinalis Wall ex Watt.
Panicum repens Linn.
P. trypheron Schult.
Paspalidium flavidum A. Cam.
Paspalam distichum Linn.
Perotis indica
Spordodus diander Beauv.
Tripogon roxbughianus Bhide.
Urochloa panicoides P. Beauv.
Amarantaceae
Allmania nodiflora R. Br.
Amaranthus viridus Linn.
Gomphrena globosa Linn.
Boraginaceae
Heliotropium panicutatum Br.
Compositae
Eclipta prostrata Linn.
Hymentherum tenupalium Dc.
Lagasca mollis Cav.
Tridax procumbons Linn.
Convolulaceae
Ipomaea reptans Poir.
Euphorbiaceae
Euphorbia pilulifera Linn.
Centinaceae
Enicostema littorale Bl.
Ltyhraceae
Ammania baccifera Linn.
Pontederiaceae
Euchornia crassipes Solms.
Typhaceae
Typha augustata Chaub. & Bory.
CULTIVATED PLANTS
Gramineae
Eleusine coracana Gaertn.
Oryza sativa L.
Pennisetum typhoideum Rich.
Saccharum officinarum Linn.
Sorghum vulgare Pers.
Triticum aestivum Linn.
Zea mays Linn.
Leguminosae
Arachis hypogeae Linn.

were recorded every three days for nymphs and daily for adults. The test was repeated for both species by releasing 100 first instar nymphs per plant, when it was found suitable for either of the species. The adults thus obtained were reared further on the same hosts for assessing their longevity and fecundity. The growth-index values were calculated by dividing the percentage of nymphs reaching adult stage by average developmental period.

2.3 *Suitability and intake of food*

Suitability of different plant species as food was assessed by recording the gain in body weight of 100 nymphs at five-day intervals. The food intake behaviour on various hosts was studied by caging two pairs of adults per leaf for 24 hr in suitable cages and collecting the honey dew excreted on a filter paper which was later treated with ninhydrin solution. The number of probing marks produced on hosts and non-host plants was determined by caging a single insect for 6 hr on individual leaves. The probing marks were stained by dipping in 1 % erythrosin solution.

2.4 *Preference of the insects for settling and oviposition*

These studies were conducted by planting selected test plants at random in a wooden flat containing puddled soil, at a spacing of 10 × 15 cm. When the plants were established, 200 five-day old adults were released inside the cages wherein the test plants had been kept. The settling behaviour of the insects was studied at regular intervals and after four days the plants were dissected to count the number of eggs laid. Similarly, the preference of fourth instar nymphs was also studied.

2.5 *Mode of food plant selection*

The role of vision was assessed by releasing adults whose eyes were closed with a thin film of dark-coloured nail polish in a cage where the most preferred food plant was kept at a distance of 30 cm. The movements of the insects were watched continuously for 6 hr.

The importance of antennae in food selection was studied by releasing adults (whose antennae were removed) on the non-host plants kept on one side of a partition cage. On the other side of the cage, suitable host plants were kept. After the insects settled on non-host plants, the partition of the cage was removed and the number of insects successfully reaching host plants was counted. Similar test was conducted with normal insects.

Role of olfaction was studied by using a five-armed choice chamber. Plant leaves were inserted into the tubes at varying lengths. Four plants were used at a time and one tube was kept blank. One hundred adults were released in the middle of the chamber and the number of insects reaching various plants and blank was recorded after 3 hr.

3. Results and discussion

3.1 *Host range*

N. virescens nymphs could not survive beyond 3 days on any of the test plants except rice (table 2). On rice, the nymphal survival was 98 % and the adult longevity was longer

Table 2. Survival of first instar nymphs and adult longevity of *N. virescens* and *N. nigropictus* on different plant species

Plant species	Percentage survival of 1st instar nymphs 3 days after caging		*N. virescens*			*N. nigropictus*		
			Adult longevity in days		Average no. of eggs	Adult longevity in days		Average no. of eggs
	N. virescens	*N. nigropictus*	Female	Male		Female	Male	
**Oryza sativa* L.	98	95	16·5	15·9	205·0	15·5	14·2	108·0
Oryza officinalis Wall ex Watt	0	93	3·2	2·4	0·0	9·6	8·6	47·4
Echinochloa colonum Linn.	0	100	3·4	3·1	5·0	12·4	10·3	64·2
Echinochloa crusgalli	0	100	3·7	2·9	11·0	13·7	9·8	82·7
Leersia hexandra Sw.	0	100	2·8	2·0	0·0	16·3	13·9	146·0
Panicum repens Linn.	0	90	1·9	1·6	0·0	7·4	7·0	26·3
Saccharum officinarum Linn.	0	90	2·8	1·9	0·0	8·5	7·4	24·5
Cynodon dactylon Pers.	0	91	2·2	1·8	0·0	8·2	7·2	0·0
Cyperus rotundus Linn.	0	0	1·6	1·1	5·0	1·9	1·5	0·0
Cyperus distans Linn. f	0	0	1·8	1·3	0·0	2·0	1·5	4·0
Cyperus difformis Linn.	0	0	1·3	1·4	3·0	1·7	1·1	0·0

* Variety TN 1.

(15·9 to 16·5 days) compared to other plant species. On the other hand, nymphs and adults of *N. nigropictus* survived on eight plant species for a longer period (7 to 16·3 days). Chu *et al* (1981) reported that ratoon rice, *Echinochloa crusgalli, Alopecurs aegualis* and *Leersia hexandra* were the best alternate food plants of *N. cincticeps* (Uhl.). An interesting aspect of the present investigation is that *N. virescens* could utilise successfully only the susceptible rice variety (TN 1) in host range tests both with first instar nymphs as well as adult insects. The high specialisation of this species (*N. virescens*) towards rice in the present investigation is contrary to the earlier conclusions made by Prasada Rao and John (1974) that *N. virescens* can feed on other plant species including dicotyledons based on the criterion of oviposition. The above criterion used by these workers seems to be not appropriate since the green leafhopper adults laid few number of eggs (table 2) even on non-host plants *viz. Cyperus rotundus* (5), *C. difformis* (3), *E. colonum* (5) and *E. crusgalli* (11). Similarly, Alam (1974) based on field observations reported hopper burn on *Leersia hexandra* by *N. virescens* while in the present studies it was found to be unsuitable as host to this species.

Since both species laid eggs even on non-host plants under confinement (table 2) and could survive around two days even under starvation (figure 1) it seems necessary to consider such aspects while determining the host status of plant species against test insect.

3.2 *Host suitability*

Host suitability for *N. nigropictus* was better on *L. hexandra* followed by *Echinochloa crusgalli, Oryza sativa* and *E. colonum* (table 3). However, *Cynodon dactylon, Panicum repens* and *Oryza officinalis* were less suitable. Though the growth-index value was 1·2 for *Saccharum officinarum*, the fecundity of the surviving insects was poor. Further the insects which fed on *L. hexandra* had the highest body weight (figure 2) compared to other host plants. The insects fed on *O. sativa* and *E. crusgalli* had almost the same weight increase after 15 days closely followed by *E. colonum*. However, insects reared on *C. dactylon* and *P. repens* showed lesser increase in body weight.

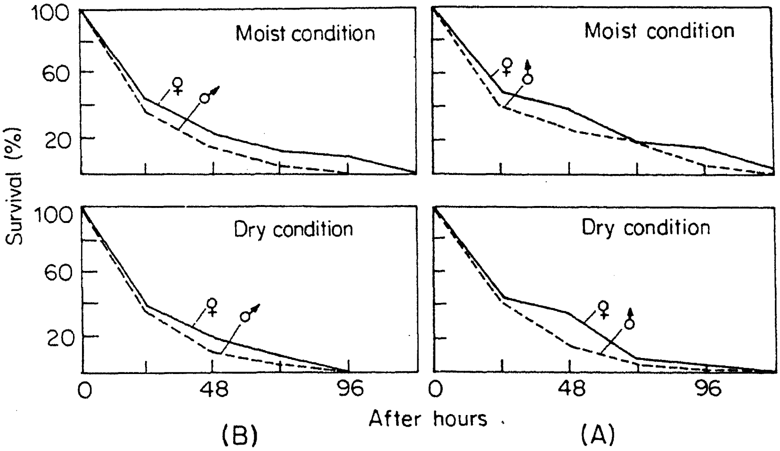

Figure 1. Survival potential of adults without food. (A) *N. virescens.* (B) *N. nigropictus.*

Table 3. Survival and development of *N. nigropictus* on selected plant species

Host plant	Percentage of survival after days			Average developmental period (days)	Average adult longevity (days)		No. of eggs laid	Growth index
	3	12	21		Female	Male		
**Oryza sativa*	98	80	50	17·8	20·5	19·0	174	2·8
Oryza officinalis	90	54	6	21·2	10·0	9·5	16	0·3
Echinochloa colonum	95	80	52	18·5	19·5	19·0	160	2·8
Echinochloa crusgalli	100	66	48	17·5	21·0	17·5	189	2·2
Leersia hexandra	100	79	65	17·2	21·2	19·5	223	3·8
Panicum repens	66	6	4	21·0	7·0	9·0	38	0·2
Saccharum officinarum	90	54	21	18·2	12.5	10·5	13	1·2
Cynodon dactylon	95	22	6	21·0	10·5	11·0	21	0·3

*Variety TN 1.

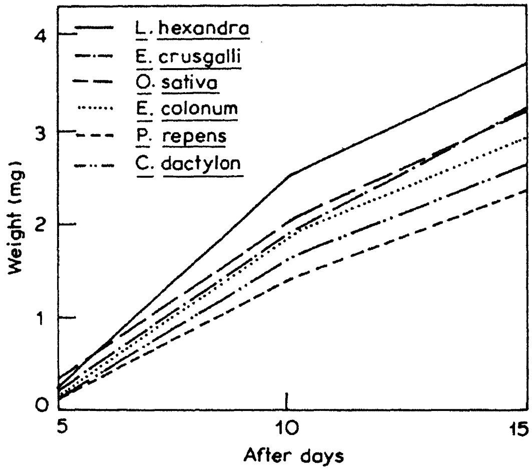

Figure 2. Extent of body weight gain by *N. nigropictus* in relation to different host plants.

Data on feeding behaviour showed that *N. virescens*, caged on host plants of *N. nigropictus*, made greater number of punctures (6·6 to 12·2) in an attempt to feed on these plants (table 4) and also excreted less honeydew thus confirming the unsuitability of these test plant species to *N. virescens*. On the other hand, *N. nigropictus* made relatively less punctures (2·8 to 6·4) and deposited more honeydew while feeding on these plant species. Similar observations were reported by Naito and Masaki (1967) for *N. cincticeps*. *Oryza sativa* for *N. virescens*, *Echinochloa colonum* and *Leersia hexandra* for *N. nigropictus* were found to be most suitable hosts for feeding. The restricted host range in case of *N. virescens* could be due to the presence of some deterrants or absence of certain stimulants needed for normal feeding in the plant species tested.

Table 4. Number of probings made by *N. virescens* and *N. nigropictus* on the leaf blades of selected plant species

	Average probings/insect/hour					
	N. virescens			*N. nigropictus*		
Plant species	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Cynodon dactylon	9·2	14·5	11·6	3·1	7·2	6·4
Echinochloa crusgalli	7·4	14·0	12·2	2·8	4·6	3·1
Echinochloa colonum	5·7	8·9	7·2	2·0	3·4	2·4
Leersia hexandra	5·0	7·6	6·6	2·0	3·1	2·8
**Oryza sativa*	2·6	4·1	3·6	3·0	6·1	4·9

*Variety TN 1.

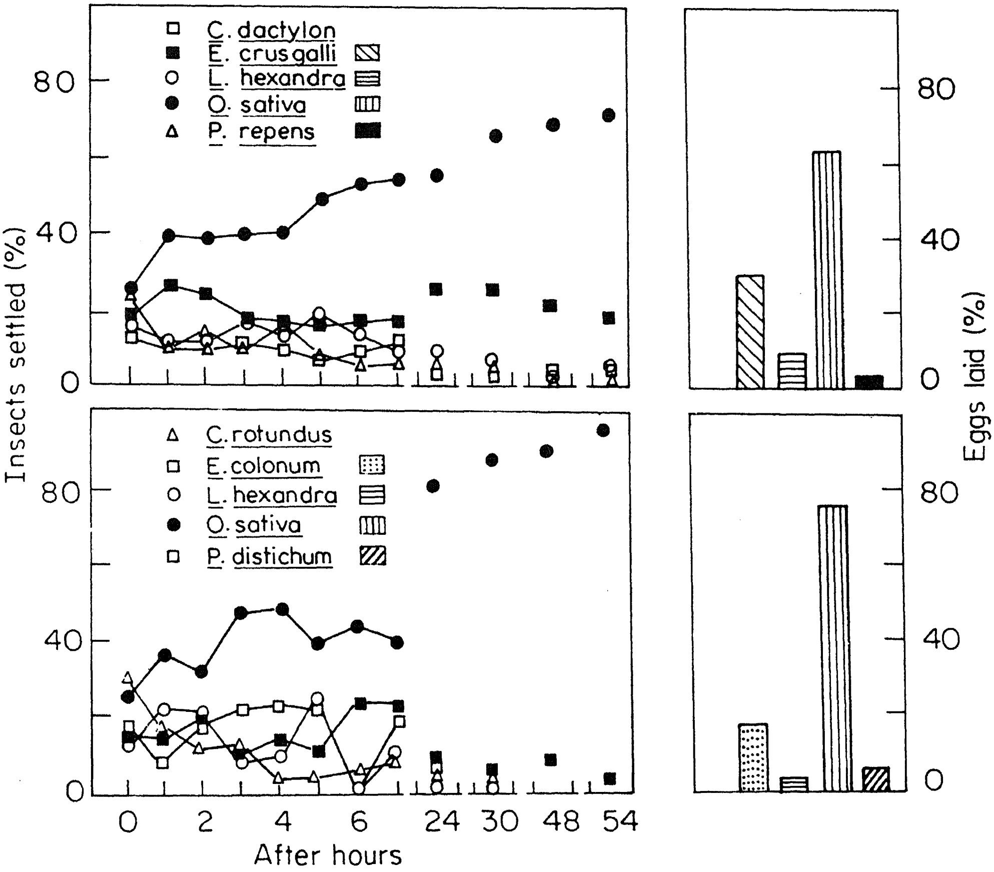

Figure 3. Preference for settling and oviposition by *N. virescens* on different plants.

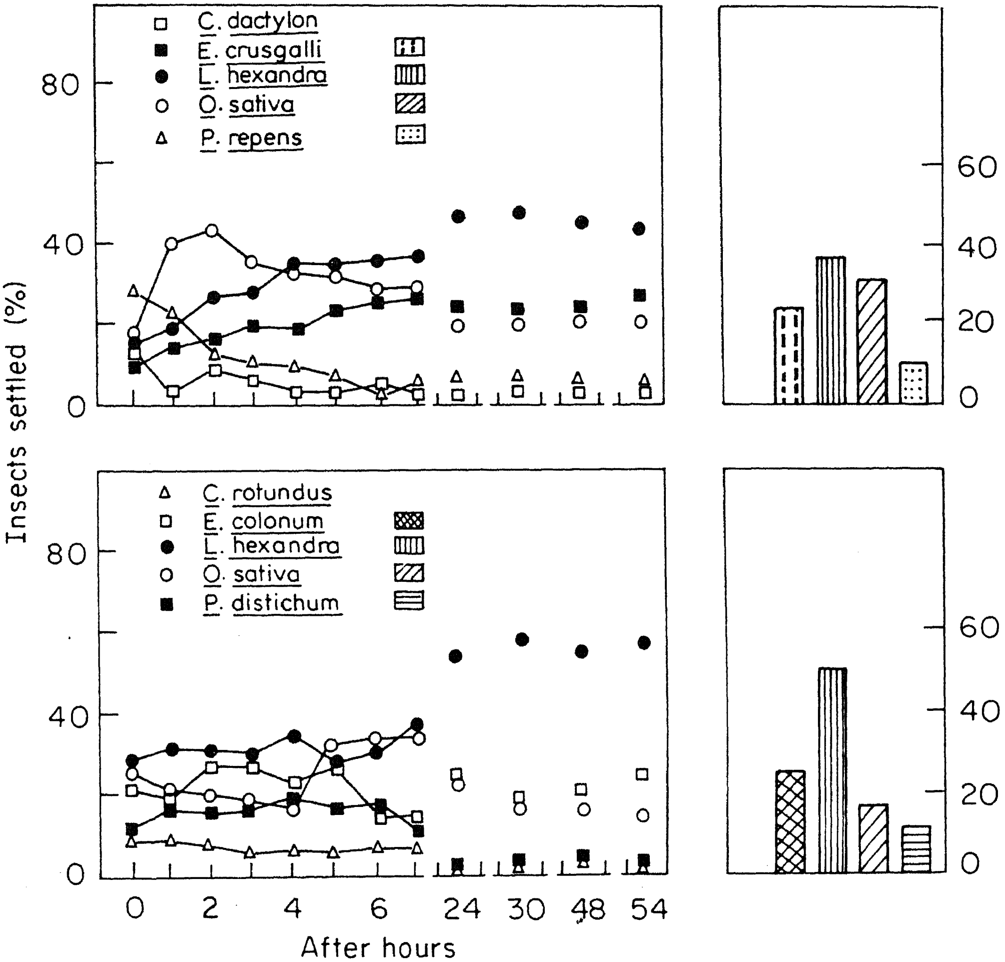

Figure 4. Preference for settling and oviposition by *N. nigropictus* on different plants.

3.3 *Host selection*

Results of multiple choice tests with *N. virescens* and *N. nigropictus* adults are presented in figures 3 and 4, respectively. As evident from figure 3, *N. virescens* was able to select rice plant, the most preferred host, within a few hours after release and also laid a larger number of eggs on it. The insects did alight on non-hosts, but little time was needed to reject such plants. However, the insects laid some eggs even on non-hosts.

Since a greater number of host plants of *N. nigropictus* were in the tests with this species, the high preference was not apparent until 24 hr. However, after this period *L. hexandra* was found to be the most preferred host by this species. In general, a larger number of eggs were also noticed on *L. hexandra* followed by *O. sativa* (TN 1), *E. crusgalli* and *E. colonum* (figure 4). In separate studies nymphs of both species were also found to locate their suitable hosts within a few hours after release.

It was observed that vision is important only for settling (table 5) and not involved in the actual selection process. However, in some other insects visual stimuli are involved in the behavioural responses before alighting. Olfaction also appeared to have no significant role in selecting suitable hosts as indicated both in the tests with antennae removed insects (table 6) and in choice chamber tests. This is further supported by probing behaviour studies where the insects made punctures even on non-host plants. Thomas (1972) also found that the beet leafhopper *Circulifer tenellus* was unable to discriminate between any preferred and non-preferred species until the feeding occurred. According to Sogawa (1973) the brown planthopper also seemed to localise the sucking tissues by random exploratory probings. Therefore, similar to many other insects, the host selection by rice green leafhoppers in the present study is of chemosensory nature and principally by gustatory stimuli.

The foregoing discussion on host specificity suggests a restricted host range of *N. virescens* and highly specialised compared to *N. nigropictus* which seems to be oligophagous. However, further critical studies are necessary in different agro-climatic zones by testing large number of plant species from the local flora to get additional information, particularly for *N. virescens* being the vector of tungro virus disease.

Table 5. Role of vision in host selection by *N. virescens* and *N. nigropictus*

	Percentage of insects settled on host plants hours after release		
Insects	3 hr	6 hr	8 hr
*N. virescens**			
Normal	15	35	89
Eyes closed	0	0	0
*N. nigropictus***			
Normal	21	47	75
Eyes closed	0	0	0

Host plants: **Oryza sativa* (TN 1) ***Leersia hexandra*

Table 6. Role of antennae in host selection by *N. virescens* and *N. nigropictus*

		Percentage of insects settled			
		With antennae		Without antennae	
Insect	Host	24 hr	48 hr	24 hr	48 hr
N. virescens	**O. sativa*	88	96	76	91
N. nigropictus	*L. hexandra*	71	90	70	79

*Variety TN 1.

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Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera)

E T HARIDASS
Entomology Research Institute, Loyola College, Madras 600 034, India

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Abstract. The harpactorine eggs, laid in clusters and covered by spumaline secretions, are distinguished from the eggs of other reduviid subfamilies by the elaborate net-work-like chorionic collar extensions and opercular outgrowths. The collar extensions may be short with spongy structure, or considerably long with honeycomb structure terminating in filamentous apices. The body of the egg has prominent follicular markings and the anterior end is marked by a distinct collar having the spermatic groove, aero-micropylar openings and the sealing bar. The chorionic collar extensions and the opercular outgrowths, along with the aero-micropylar systems and the inner aerostatic layer, efficiently transport atmospheric oxygen to the interior of the eggs. The ultrastructure of the chorion, the operculum and the aero-micropylar system of 5 species of harpactorine bugs from Southern India is reported.

Keywords. Reduviidae; Harpactorinae; egg; chorion; operculum.

1. Introduction

The harpactorine eggs are considered unique in many respects among Reduviidae and have received considerable attention from many workers (Kershaw 1909; Readio 1926; Odhiambo 1956; Southwood 1956; Edwards 1962; Cobben 1968; Cobben and Henstra 1968; Miller 1953, 1971; Salkeld 1972; Hinton 1981). Though there is uniformity in the general organisation within the subfamily, there definitely exist many distinct variations in the egg structure of different species. An attempt is presently made to study these variations in the eggs of 5 harpactorine bugs collected from Southern India.

2. Materials and method

Eggs of the following species were studied:

(i) *Sycanus collaris* Fabricius, (ii) *Rhinocoris fuscipes* (Fabricius), (iii) *Harpactor pygmaeus* Distant, (iv) *Endochus inornatus* Stal, (v) *Lophocephala guerini* Laporte.

Methods involved in the study of these eggs have been described elsewhere (Haridass 1985b).

3. Observation

3.1 *Oviposition*

All harpactorines oviposit clusters of numerous eggs gluing them with cementing materials to the under surfaces of boulders, stones, or on the barks of trees or shrubs (Haridass 1985a). Oviposition is very regular, except for the first 3–4 eggs, in kind of chevron sequence, where successive half-rows of eggs are deposited from the margins towards the middle line of the egg mass (figure 1A). Four to six egg masses are laid by a single female, there being no parental care in any of the species studied.

3.2 *Structure of egg*

3.2.1 *Colour, size and shape*: Eggs of harpactorine species are more or less uniform in shape, the main part of the egg is cylindrical with rounded posterior end with distinct

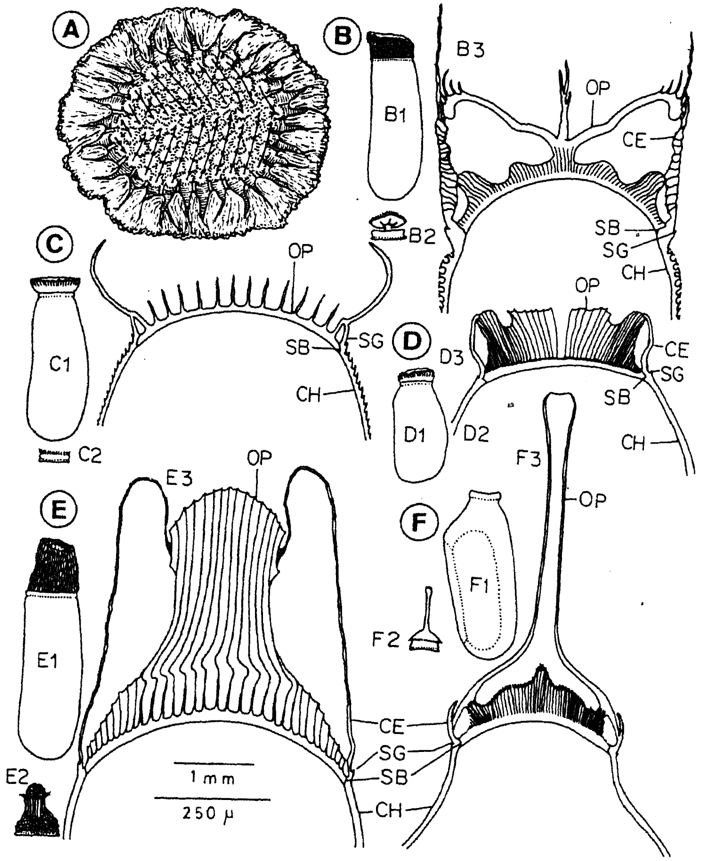

Figure 1. Eggs of Harpactorinae. **A.** *S. collaris*. Egg mass and ovipositional sequence (as shown by arrows). **B.** *S. collaris*. Entire egg (B1), operculum (B2), and L.S. of anterior end of the egg (B3). **C.** *R. fuscipes*. Entire egg (C1), operculum (C2), and L.S. of anterior end of the egg (C3). **D.** *H. pygmaeus*. Entire egg (D1), operculum (D2), and L.S. of anterior end of the egg (D3). **E.** *L. guerini*. Entire egg (E1), operculum (E2), and L.S. of anterior end of the egg (E3). **F.** *E. inornatus*. Entire egg (F1), operculum (F2), and L.S. of anterior end of the egg (F3). Scale: Entire egg and operculum = 1 mm. L.S. of the anterior end of the egg = 250 μm. (CE, Chorionic extensions; CH, Chorion; OP, Operculum; SB, Sealing bar; SG, Spermatic groove).

concavity and convexity, corresponding respectively to the dorsal and ventral sides of the fully developed embryo. The eggs of *Endochus inornatus* alone have typical bottle shape, the wide body having narrow collar and operculum (figure 1F1). In *Sycanus collaris*, the eggs are cylindrical, and are dark and honey coloured, including the operculum. The cylindrical eggs of *Harpactor pygmaeus*, *Rhinocoris fuscipes* and *E. inornatus* are dark brown, but their chorionic collar extensions and the operculum are white. The eggs of *Lophocephala guerini* are also cylindrical with white operculum and pale yellowish body (figures 1B1, C1, D1, E1). The exposed parts of all the eggs are always darker than those parts which are in contact with the adjacent eggs. The sizes of the eggs of different species are given in table 1.

3.2.2 *Body of eggs:* All harpactorine eggs have their main body marked by follicular pits but these are greatly obscured by the application of the cementing material at the time of oviposition. While the pits are prominent throughout the chorion in *R. fuscipes* (figure 2B), those of *S. collaris* are prominent only on the upper one third of the body and become gradually shallower towards the posterior end (figure 2A). In *H. pygmaeus*, *E. inornatus* and *L. guerini* the follicular pits are very shallow, demarcated only by hexagonal ridges. In all the eggs, the follicular pits are prominent in exposed areas than on those parts which are in actual contact with other eggs.

The chorion of the eggs is composed of an outer exochorion merging imperceptably with the inner endochorion and the relative thickness of these two layers vary, not only among eggs of the same batch, but also in the same egg at different regions. The chorion is thicker and tougher in the eggs situated at the perimeter of the egg mass than those in the middle and in any given egg, the chorion is thicker near the neck region (20–26 μm) than in the rest of the body (2–3 μm). The chorion of the eggs which are in contact with each other, remains thin and flexible, the reduction in thickness of exochorion being the cause for it. In areas where there are prominent follicular pits, the boundaries of these pits arise as extensions of the exochorion. The innermost part of the endochorion shows the aerostatic layer (figures 4B, C), varying between 0·5–6 μm in thickness in different eggs. This layer is separated from the endochorion by supporting pillar-like

Table 1. Measurements and the aero-micropylar numbers in Harpactorine eggs.

	Eggs (n = 10)				Number laid/female (n = 5)	
Species	Length (mm)	Width (mm)	Aeropyles	Micropyles	Eggs	Egg batches (average female longevity)
S. collaris	1·85–1·90 (1·88)	0·66–0·69 (0·68)	116–134 (126)	9–12 (11·4)	52–398 (234·1)	3–4 (84 days)
R. fuscipes	1·58–1·63 (1·61)	0·58–0·63 (0·61)	92–118 (104)	4–6 (5·2)	60–192 (90·4)	5–6 (76 days)
H. pygmaeus	1·08–1·12 (1·10)	0·54–0·57 (0·55)	158–181 (176)	4–7 (6·6)	28–160 (81·6)	5–6 (69 days)
E. inornatus	2·18–2·22 (2·20)	0·84–0·87 (0.86)	89–107 (98)	6–8 (7.2)	40–178 (92.4)	4 5 (80 days)
L. guerini	2·30–2·35 (2·33)	0·81–0·84 (0·83)	133–154 (148)	8–10 (9·7)	32–210 (142·3)	3–4 (54 days)

The figure in parentheses indicates mean values.

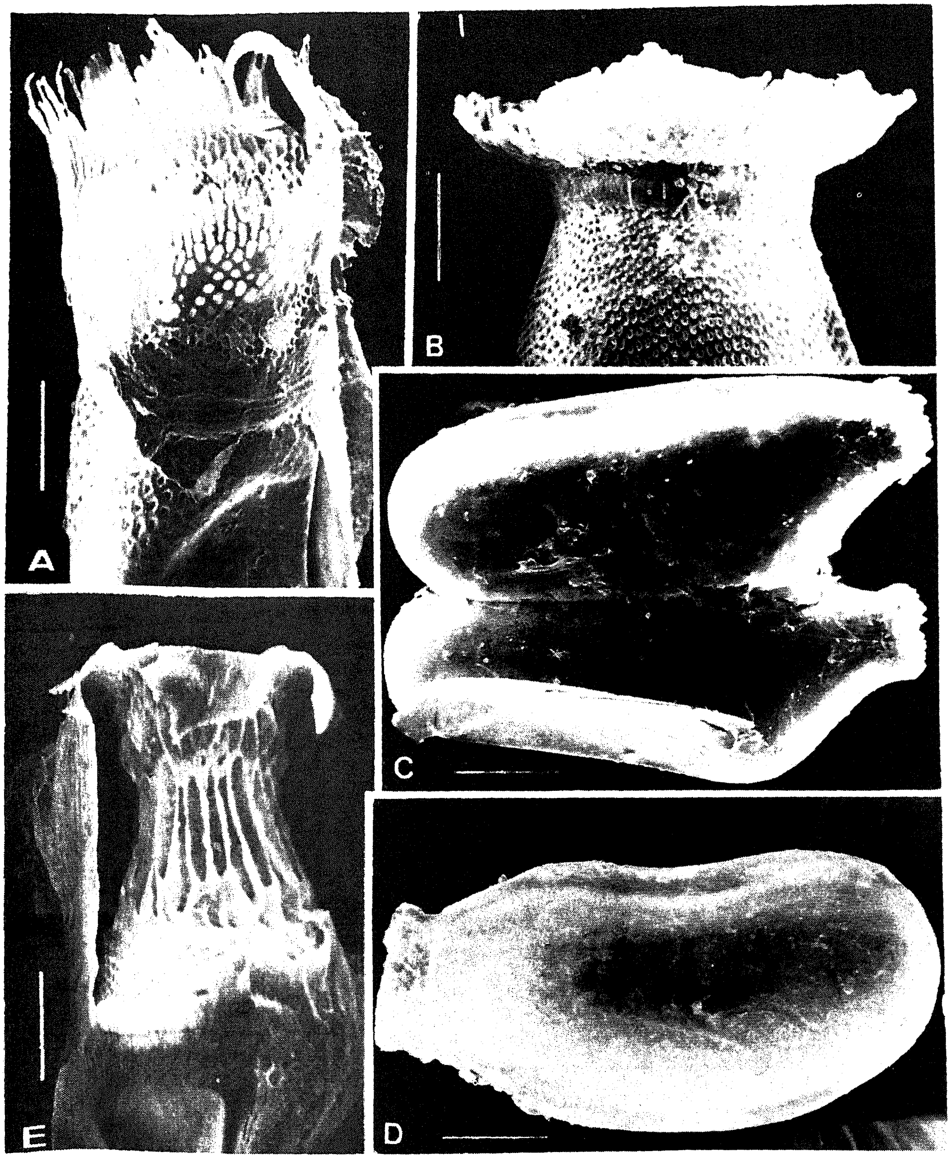

Figure 2. Scanning electron micrographs of Harpactorine eggs. **A.** *S. collaris*. Anterior part of the egg with operculum (Scale = 150 μm). **B.** *R. fuscipes*. Anterior part of the egg (Scale = 176·5 μm). **C.** *E. inornatus*. Entire eggs (Scale = 303 μm). **D.** *H. pygmaeus*. Entire egg (Scale = 169 μm). **E.** *L. guerini*. Anterior part of the egg with operculum; chorionic collar extension partly removed (Scale = 139·5 μm).

struts exhibiting a polygonal arrangement, corresponding to the chorionic follicular pits. The aerostatic layer starts immediately below the sealing bar and extends all around the inner aspects of the egg and is distinctly absent in the operculum. At the point of origin where it receives all the inner aeropylar openings, the aerostatic layer is wider than in the rest of the chorion (figure 4B).

3.2.3 *Chorion:* As in other reduviids, the anterior part of the harpactorine egg is characterised by a distinct neck or collar which is further distinguishable into an outer collar rim composed of exochorion and an inner sealing bar wholly formed of endochorion. While the outer apical margin of the collar has a very shallow and narrow spermatic groove all around (figures 1A-F; 4A, D), its inner side facing the lumen of the egg, is marked by numerous vertical and alternating grooves and ridges, constituting the sealing bar. These grooves and ridges receive similar grooves and ridges of the opercular rim, enabling the operculum to fit snugly and correctly into the collar. The grooves and ridges of different eggs may be oriented vertically, obliquely, or horizontally to the long axis of the egg (figures 4B, E, F, G).

The anterior extremes of the endochorionic part of the collar, between the spermatic groove and the sealing bar, is produced into a net-work like extensions variously modified in different species. In *H. pygmaeus* they are very short and extend only upto the level of the opercular extensions (figures 1D1, D3; 2D). The collar extensions of *E. inornatus* extend only to the basal part of the long operculum and they show a spongy appearance (figures 1F1, F3; 4F). The chorionic collar extensions of *R. fuscipes* extend well beyond the upper limits of the opercular extensions, projecting away from the central axis of the egg as highly porous continuous sheet (figures 1C1, C3; 2B; 4D, E). In *S. collaris* the collar extensions project far beyond the upper limits of the opercular extensions and have the honey-comb structure basally, but terminating as free elongated filamentous structures distally (figures 1B1, B3; 2A; 3B; 4A). The collar extensions of *L. guerini* are equally as long as those of the operculum. At the distal end this highly porous continuous sheet-like extension with distinct polygonal cells, is turned inwards to join the opercular apical rim (figures 1E1, E3; 2E; 3C; 4G). This kind of union between the collar extensions and the extensions from the operculum is also common in all the eggs, but the actual point of union may be at the base or in the middle or at the apex of the operculum (figures 1B-F). During eclosion these connections are snapped all around, enabling the embryo to escape from the egg by pushing the operculum like the lid of a jar.

3.2.4 *Aeropyles and micropyles:* In spite of the variations in the chorionic collar extensions and the opercular structures, the aeromicropylar system of harpactorine eggs presents a uniform pattern in all the species presently studied. The external aeropylar openings are situated in the space between the operculum and the chorionic extensions, on a ring of spongy area on the upper chorionic rim surface above the sealing bar (figures 4B, E). The number of aeropyles vary from 98–176 in different species examined (table 1). The external aeropylar openings continue from the spongy area as canals of 1·5–3·0 μm diameter, extend downwards through the endochorion, pass behind the sealing bar and open into a circular or oval space on the inner aerostatic layer commencing immediately below the sealing bar (figures 4B, G). The aeropylar canals and openings thus establish a pathway between the aerostatic layer and the air space between the operculum and chorionic collar extensions. In no case the external aeropylar openings are noticed on the outer surfaces of the chorion.

The micropyles are easily distinguished from the aeropyles by their wider external openings (8–12 μm) and also by their position on the outer surfaces of the chorion above the neck regions, very near to the point of origin of the collar extensions (figures 4A, D). The micropyles penetrate through the thin exochorion and follow the

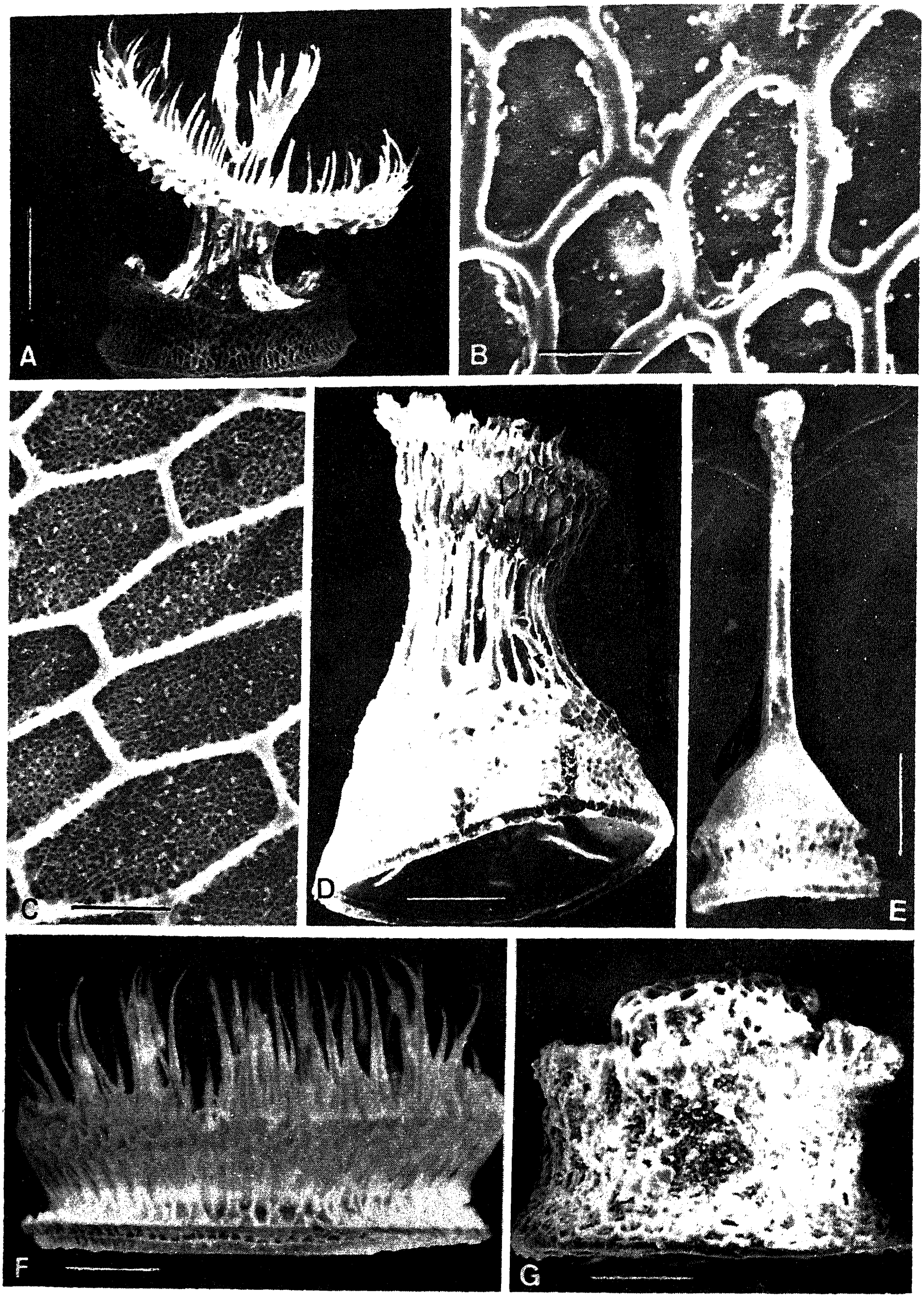

Figure 3. Scanning electron micrographs of Harpactorine eggs. **A.** *S. collaris.* Lateral view of the operculum (Scale = 117·6 μm). **B.** *S. collaris.* Enlarged view of the polygonal cells of chorionic collar extension (Scale = 14·5 μm). **C.** *L. guerini.* Enlarged view of the polygonal cells of chorionic collar extension (Scale = 24·4 μm). **D.** *L. guerini.* Lateral view of the operculum (Scale = 112·7 μm). **E.** *E. inornatus.* Lateral view of the operculum (Scale = 83·3 μm). **F.** *R. fuscipes.* Lateral view of the operculum (Scale = 55·8 μm). **G.** *H. pygmaeus.* Lateral view of the operculum (Scale 53·8 μm).

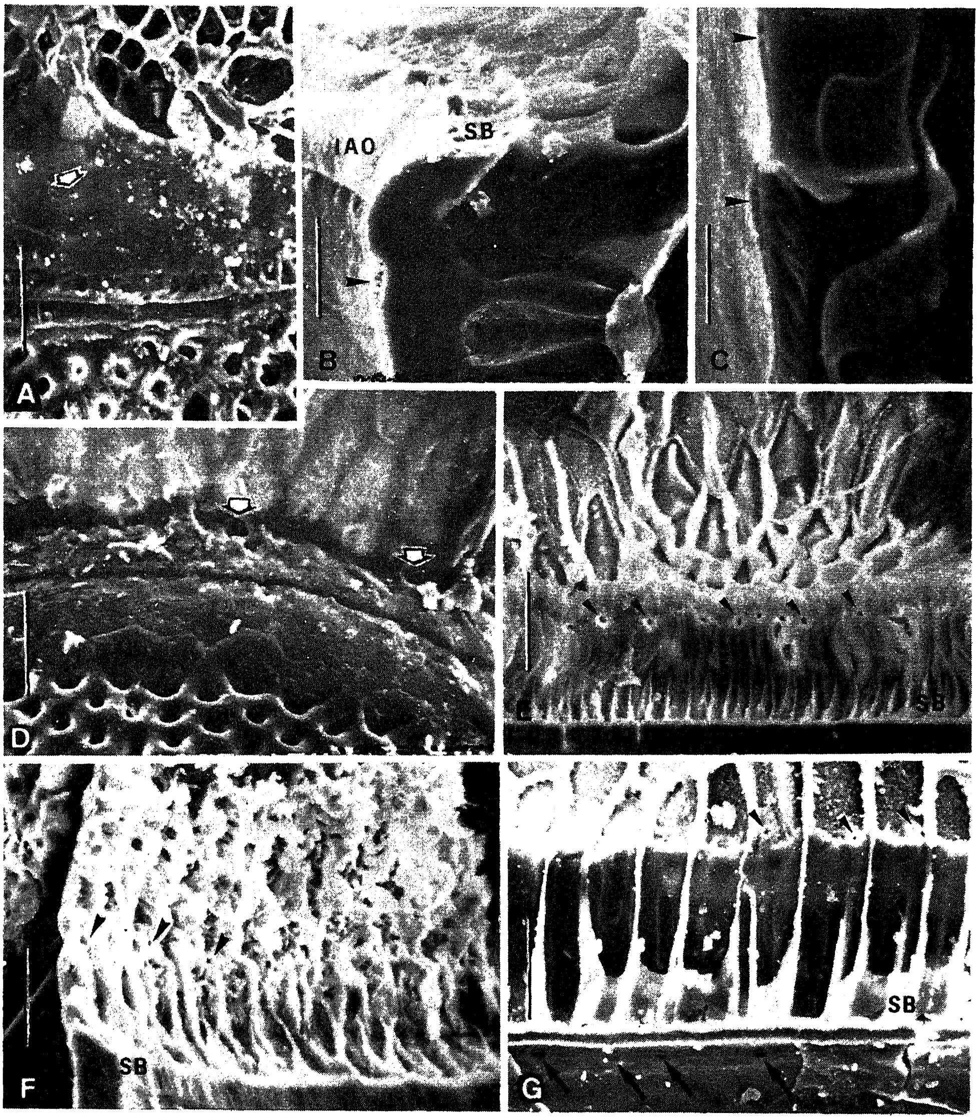

Figure 4. Scanning electron micrographs of Harpactorine eggs. **A.** *S. collaris.* Chorionic collar rim. Arrow points to the outer micropylar opening (Scale = 42·1 μm). **B.** *S. collaris.* L.S. of the chorion near collar region. Arrow indicates the beginning of the inner aerostatic layer; SB = Sealing Bar; IAO = Inner Aeropylar opening (Scale = 16 μm). **C.** *R. fuscipes.* L.S. of chorion in the middle of the body. Arrow indicates the aerostatic layer. (Scale = 7 μm). **D.** *R. fuscipes.* Chorionic collar rim showing spermatic groove and micropyles (Arrows) (Scale = 21 μm). **E.** *R. fuscipes.* Chorionic collar inner view showing sealing bar and outer aeropylar openings (Arrows) (Scale = 38·6 μm). **F.** *E. inornatus.* Chorionic collar inner view showing sealing bar and outer aeropylar openings (Arrows). (Scale 21·33 μm). **G.** *L. guerini.* Chorionic collar inner view showing sealing bar, outer aeropylar openings (small arrows) and inner aeropylar openings (long arrows). (Scale = 30·8 μm).

same course like aeropyles in the endochorion. Inner to the aerostatic layer, the micropylar canals terminate as a bent duct or groove into the lumen of the eggs, the terminal bent portions exhibiting a clockwise arrangement when viewed from the anterior end. The number of aeropyles and micropyles in different species is tabulated (table 1).

3.2.5 *Operculum:* The operculum of harpactorine eggs exhibits great structural diversity in the different species examined. The part of the operculum that fits into the collar of the egg through a distinct sealing bar, is of uniform nature in all the eggs, being ring-like with alternating vertical grooves and ridges. The upper part of the operculum, on the other hand, varies considerably. In *R. fuscipes* the operculum is disc-like with numerous upright filamentous extensions from its upper surface (figures 1C3; 3F), the peripheral filaments being connected to the chorionic collar extensions all around. In *H. pygmaeus* the operculum is cylindrical with a column of honey-comb area at the apex with central part pushed down into a deep cavity. The lumen of this cavity and the air spaces of the honey-comb area extend deep into the opercular base. The peripheral edge of the cylindrical operculum is united with the chorionic collar extensions all around (figures 1D3; 3G). In *E. inornatus* the operculum is peculiar; the highly porous disc-like basal rim is united with the collar extensions all around and the conical part above the basal disc is protracted into a bulb with a large terminal opening. The conical part of the operculum has numerous filamentous projections inside (figures 1F3; 3E). In *S. collaris* the basal part of the operculum is raised into a cylinder of numerous honey-comb cells, the cavities of these cells penetrating the opercular base obliquely. From the centre of this cylinder arises a vertical pillar supporting an arched disc apically, the periphery as well as the central concavity of this disc is produced into numerous filaments. Some of these peripheral filaments are united with the chorionic collar extensions (figures 1B3; 3A). In *L. guerini* the operculum is conical with many honey-comb cells, the central part of this cone is protracted distally to end in a blunt convex apical part. The basal rim of this blunt end is connected with the chorionic collar extensions (figures 1E3; 2E; 3D). In spite of the structural diversities, the operculum of all the species examined possessed numerous pore canals, arising from the air spaces between the honey-comb cells or from the interfilamentar spaces and penetrating the endochorion to open finally into the lumen of the egg through the concave opercular base. The inner aerostatic layer, distributed all over the interior of the egg, is distinctly absent in the operculum.

4. Discussion

Despite the general similarities with other families of Cimicomorpha, the eggs of Reduviidae have been shown to be unique in the structure of the operculum, aero-micropylar system and chorionic collar. The eggs of Piratinae (Haridass 1985b) and Harpactorinae stand out as most characteristic among this family and in fact, the latter has been the most widely studied of all the subfamilies of Reduviidae (Kershaw 1909; Readio 1926; Bose 1951; Miller 1953, 1971; Odhiambo 1956; Southwood 1956; Cobben 1968; Cobben and Henstra 1968; Salkeld 1972; Hinton 1981; Ambrose and Livingstone 1979). Laying hexagonal clusters of spumaline covered eggs and transforming such masses into a kind of ootheca is common in most harpactorinae, though oviposition of the eggs singly or in a single row or in parallel rows are also known in some species (Miller 1971). The significance of this reproductive strategy is to protect the eggs from the ravages of hymenopterous and other egg parasites and predators. Though parental care has not been observed in any species studied presently, harpactorine females (Edwards 1962) or males (Bequaert 1912; Odhiambo 1956) have been reported to exhibit brooding behaviour, affording additional protection to the eggs.

The harpactorine eggs, in a majority of cases, are laid in a crowded manner and in habitats that are well above the ground level, with easy access to the atmospheric oxygen as well as shielded from the danger of getting drowned in water during rainy seasons. Hence, numerous but the simple aeropyles, along with the aerostatic layer seen all around the interior of the eggs, and the highly porous chorionic collar extensions, provide an efficient respiratory system. The plastron respiration, so well developed due to follicular modification in eggs that are liable for submersion in water for considerable periods of time (Hinton 1981; Haridass 1985b), is totally wanting in these eggs. The total absence of the aerostatic layer in the operculum is compensated by the development of numerous pore canals in this region enabling the passage of atmospheric oxygen to the interior of the eggs.

The micropylar number in all the species presently studied varied from 4–12 and this falls within the typical number of 15 suggested for reduviids (Cobben 1968). By virtue of their location on the outer surfaces of the chorionic collar, some of the micropyles get covered with cementing secretions from the female, but a great number of these openings still remain fully exposed. It is possible, therefore, that the micropyles can subserve respiratory function also.

Inspite of the marked variation in the opercular structure, the Harpactorinae presents more or less a uniform pattern, like many species considered typical of this subfamily, in the structure of the aero-micropylar system, inner aerostatic layer, chorionic collar extensions etc., of the egg, as well as in the ovipositional behaviour of the female. Based on these characters it is possible to identify the eggs of Harpactorinae easily from those of other reduviid subfamilies. But the examination of egg characters of a large number of species is required in order to assign the variation of egg characters for the identification of Harpactorinae, atleast at the generic level.

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Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions

K VENKATARAMAN* and S KRISHNASWAMY

Department of Environmental Biology, School of Biological Sciences, *School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai 625 021, India

MS received 29 July 1985

Abstract. The longevity of *Daphnia similis* Claus is more in pond water (57 days) than in modified Banta's medium (38 days) and the preadult instar number varied from 3–5. *Daphnia cephalata* King, however shows no variation in longevity or preadult instar number when cultured in both the media. The rate of egg production of both the species were higher in modified Banta's medium than in pond water. The differences in the mean number of eggs per instar of *Daphnia similis* and *Daphnia cephalata* in two different culture media are statistically significant ($P < 0.005$; ANOVA). The variation in growth at each instar for *Daphnia similis* under two different culture media are statistically significant ($P < 0.005$; ANOVA) but for *Daphnia cephalata* it is not significant.

Keywords. *Daphnia similis*; *Daphnia cephalata*; modified Banta's medium; pond water; longevity; egg production; body size.

1. Introduction

Daphnids are one of the fish feed organisms which augment fish production. Keeping this in view, laboratory studies on aquatic microcrustaceans with special reference to daphnids have received the attention of many scientists in India (Navaneethakrishnan and Michael 1971; Bhanot and Vass 1976; Venkataraman 1981, 1983; Venkataraman *et al* 1985). Earlier studies on the life span, instar duration, egg production, growth and embryonic development of individual tropical species of Cladocera have been made using only pond water (Murugan and Sivaramakrishnan 1973, 1976; Murugan 1975; Murugan and Job 1981; Venkataraman and Krishnaswamy 1985). Since most of these studies on different species of Cladocera were made using pond water as a culture medium, it was felt that information on the effect of modified Banta's medium (equal mixture of 50 gm of cow-dung, goat-dung and pond soil in 1000 ml) on the longevity, instar number and duration, growth and egg production would be of great significance, since food is a factor these organisms have to reckon within their environment. Hence a further study of two co-occurring species, *Daphnia similis* Claus and *Daphnia cephalata* King in the modified Banta's medium and pond water was made. Such studies on life history under laboratory conditions are important for understanding mass culture and zooplankton productivity studies.

2. Material and methods

Effect of modified Banta's medium on the life history of two species of *Daphnia* were studied by collecting *D. similis* and *D. cephalata* from a seasonal pond near University campus (Lat.: 9° 53′ N; Long.: 78°E) and were acclimated to the laboratory temperature (28–30°C). Gravid females of the above species were isolated from the collection and were reared in modified Banta's medium and pond water for two days. Two sets of 15 just born neonates were separated from each species and were reared in already prepared media and pond water. The method used by Venkataraman (1981, 1983) was followed to study the life history. The number and duration of instars, number of eggs per brood, increase in length of the animal and total life span were recorded. Values obtained at different experimental conditions were tabulated to findout the differences in egg production (Analysis of variance; Zar 1974), size at birth, average life span, number of instars, instar at initial reproduction (primiparous instar), total number of eggs produced and size at death.

3. Results and discussion

The longevity of daphnids cultured in the laboratory are estimated and expressed as the mean duration of life (table 1). The mean life span for *D. similis* in pond water is 57 days which is nearly double the value obtained for animals cultured in modified Banta's medium (36 days). The longer duration in pond water is probably due to the low food quality and quantity and the short life span in modified Banta's medium can be attributed to the presence of rich organic substances. This appears to support the view that less food increases the life span. The life span of *D. cephalata* however shows no variation in both the media (figure 1).

A review of literature shows that the survival value was higher at low food levels (Ingle *et al* 1937; Arnold 1971; Venkataraman 1983). Vijverberg (1976) found relatively higher longevity values for poorly fed *D. hyalina* (64 days) than the well fed (43 days). Contrary to the above mentioned observations, Venkataraman *et al* (1985) found a higher longevity for *D. similis* cultured in pond water with rice bran (31, 38, 33 days in 250, 500 and 1000 mg/l of rice + pond water) than in pond water alone (20 days).

Thus, from the above mentioned studies it is clear that longevity is not only dependent on food level (Ingle *et al* 1937; Vijverberg 1976) but also on food type (Arnold 1971; Venkataraman *et al* 1985), temperature (MacArthur and Baillie 1929; Ringleberg 1970; Venkataraman and Job 1980) and on reproductive efforts (Schwartz

Table 1. Comparison of mean values of longevity, total number of eggs, number of preadult and adult instars, maximum growth increment and size at death of *D. similis* and *D. cephalata* cultured in pond water and modified Banta's medium.

Experimental conditions	Name of the species	Longevity days	Total number of eggs	Number of preadult instar	Number of adult instars	Maximum growth increment	Size at death
Pond water	*D. similis*	57	110	4	30	0·339(2)	2·48
	D. cephalata	37	53	5	21	0·213(3)	3·14
Modified Banta's medium	*D. similis*	36	214	5	27	0·345(4)	2·61
	D. cephalata	38	124	5	21	0·277(3)	3·33

Number of instar in parentheses.

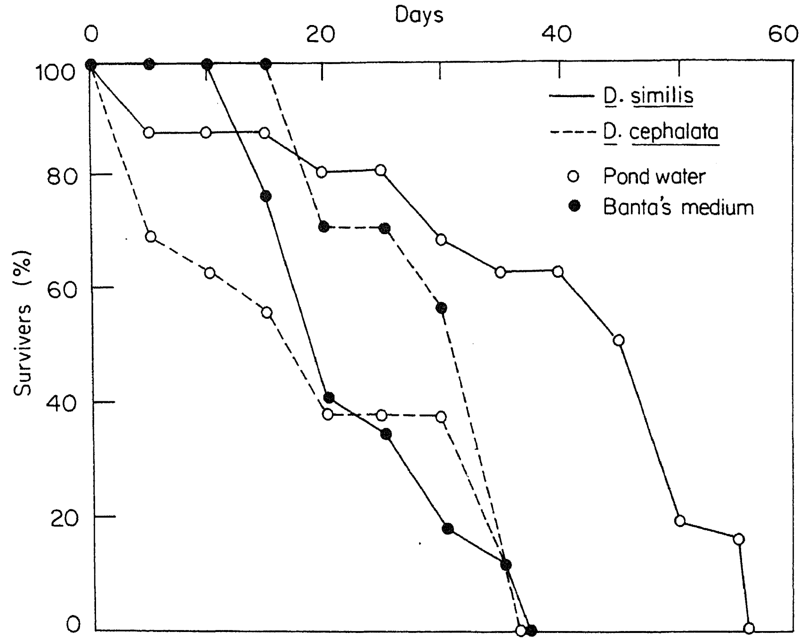

Figure 1. Percentage of survivers per day of *D. similis* and *D. cephalata* under different experimental conditions.

and Ballinger 1980). The reproductive efforts occurring frequently at each instar may stress on the organism to the extent of decreasing its life span. In the present study, *D. similis* which has a higher longevity with less number of eggs, and lesser longevity with high reproduction rate is apparent (table 1). However, the same number of days of longevity for *D. cephalata* in both the media needs a few other experiments to explain.

The number of preadult and adult instar of *D. similis* vary between 4–5 and 27–30 respectively (table 1). *D. cephalata* however, shows any variation in number of preadult instars. Among Daphnidae both variable and constant number of preadult and adult instar have been reported by many authors (Anderson 1932; Anderson *et al* 1937; Anderson and Jenkins 1942; Ingle *et al* 1937; Banta *et al* 1939). From the available literature on temperate and tropical Cladocera it is difficult to decide whether the constancy and variable number of juvenile instars represent different strategies which have evolved to suit the conditions of existence of a species and even geographically isolated populations of the same species or whether it is due to the culture conditions in the experiment.

The mean number of eggs per adult instar of *D. similis* and *D. cephalata* under two different culture media are shown in figure 2. There are two key factors which have a direct correlation with the number of eggs in the brood chamber: the size of the mother (Anderson *et al* 1937; Green 1954, 1956; Richman 1958) and the food concentration (Ingle *et al* 1937; Ryther 1954; Green 1956). Other factors which may also influence the number of eggs per female are the oxygen concentration, pH (Fox *et al* 1951) and temperature (Vijverberg 1980; Venkataraman and Job 1980).

In the present study oxygen and temperature were of no concern. Oxygen

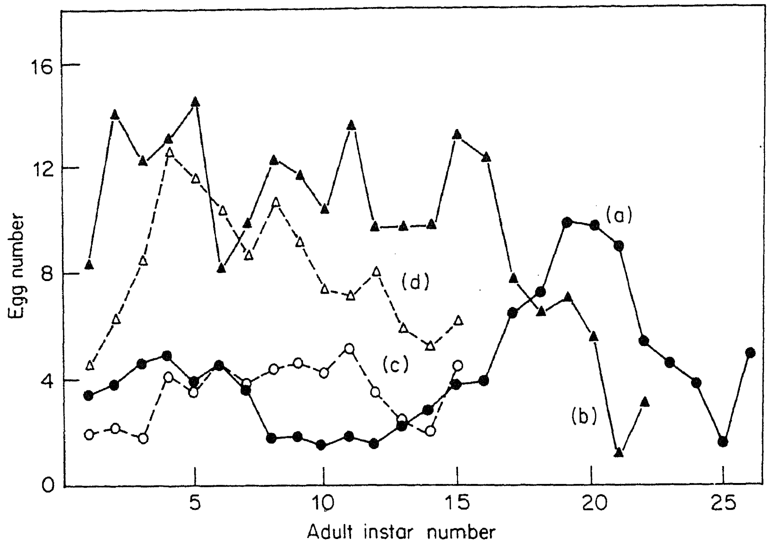

Figure 2. Mean number of eggs per brood in relation to adult instar number in *D. similis* (**a**, **b**) and *D. cephalata* (**c**, **d**) in pond water (**a** and **c**) and modified Banta's medium (**b** and **d**).

concentration limits egg production only at very low concentration (Green 1956) which did not occur in the culture and temperature was nearly constant for the two culture media.

The mean total number of eggs produced by *D. similis* when cultured in pond water is 110 in a life span of 57 days which is almost twice the number of eggs produced (213 eggs in a life span of 37 days) in modified Banta's medium. Likewise, *D. cephalata* when cultured in pond water produced a mean total number of 53 eggs in a life span of 37 days which is half of that when cultured in modified Banta's medium (124 eggs in a life span of 38 days). The differences in the mean number of eggs per adult instar of *D. similis* and *D. cephalata* in two different culture media are statistically significant (table 2). A similar situation with significant difference in egg production when the amount of food supply is more has already been reported for *D. similis* (Venkataraman *et al* 1985).

It is observed that *D. similis* cultured in pond water has a low rate of egg production ($a = 4{\cdot}226$) whereas in modified Banta's medium the rate of egg production is higher ($a = 10{\cdot}234$) (figure 3). Likewise, the rate of egg production for *D. cephalata* in pond water is ($a = 3{\cdot}84$) less than when cultured in modified Banta's medium ($a = 8{\cdot}52$) (figure 4). These results are in agreement with those of many authors who showed the existence of a positive correlation between the changes of individual fecundity and food concentrations (Edmondson 1962; Lampert 1978; Hebert 1978; Schwartz and Ballinger 1980; Venkataraman *et al* 1985).

The mean size of *D. similis* and *D. cephalata* at different culture media has been plotted against instar number (figure 5). In the present study the growth increment appears to be more during the early phase of the life cycle and at the onset of reproduction there is only a gradual increment. This confirms the already expressed view that rapid preadult growth seems to be a common feature for cladocerans irrespective of differences in experimental conditions (Anderson 1932; Wegelenska 1964; Vijverberg 1980; Venkataraman 1983).

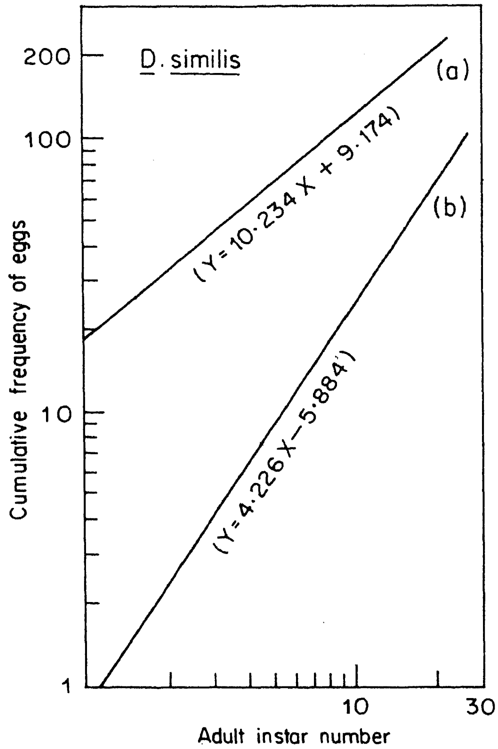

Figure 3. Cumulative frequencies of eggs against adult instar numbers of *D. similis* under two different culture media. (**a**) Modified Banta's medium; (**b**) pond water.

Table 2. Analysis of variance: Differences in egg production and growth of *D. similis* and *D. cephalata* in pond water and modified Banta's medium under laboratory conditions (28–30°C)

	Name of the species	*df*	*F*	*P*
Egg production	*D. similis*	1, 45	35·80	< 0·0005[a]
	D. cephalata	1, 28	48·96	< 0·0005[a]
Growth	*D. similis*	1, 55	8·10	< 0·01[b]
	D. cephalata	1, 40	1·12	< 0·10[c]

[a] Statistically highly significant.
[b] Statistically significant.
[c] Statistically not significant.

From the results it is observed that modified Banta's medium which contains rich organic matter may enhance the growth rate and the maximal size of *D. similis* than in pond water. Hebert (1978) found that the size reached by individuals of a particular species depends upon their food supply and when the food supply is meagre, adults cease to grow on reaching maturity and when food is abundant, growth continues throughout life. The variations in growth at each instar for *D. similis* under two

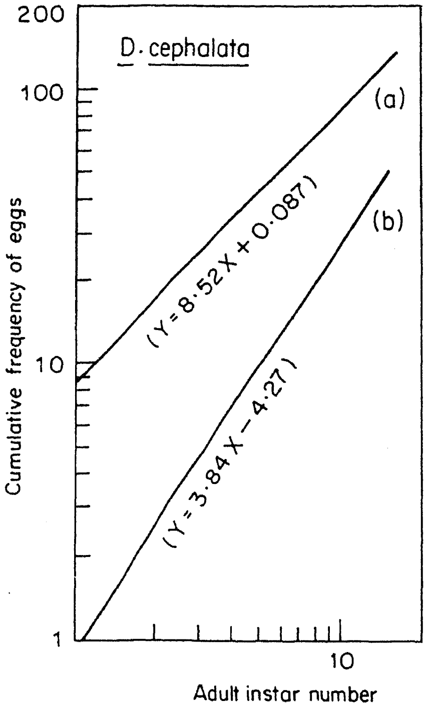

Figure 4. Cumulative frequencies of eggs against adult instar numbers of *D. cephalata* under two different culture media. (**a**) Modified Banta's medium; (**b**) pond water.

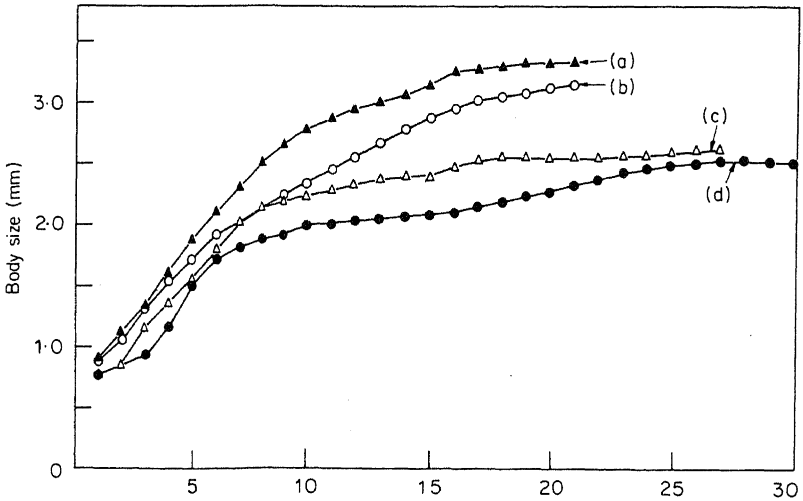

Figure 5. Relationship between mean body length and instar number of *D. cephalata* (**a**–**b**) and *D. similis* (**c**–**d**) in pond water(**b** and **d**) and modified Banta's medium (**a** and **c**).

different culture media are statistically significant (table 2). *D. cephalata*, however shows any significant variation in size at each instar under the two different culture media. Further studies on this may give an explanation for the same.

Thus, among the two species studied, *D. similis* shows higher longevity, significant difference in egg production and growth than *D. cephalata* under two different culture media. Secondly, modified Banta's medium is more nutritious for both the species, since the egg production is two fold higher than in pond water.

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Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes

A TRIVENI and P R RAO

Department of Zoology, Osmania University, Hyderabad 500007, India

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Abstract. Electrophoretic analysis of the lactate dehydrogenase isozyme patterns of several tissues of *Heteropneustes fossilis* and *Clarias batrachus* indicated that in addition to the LDH A and B genes found in these species the liver tissue of *Clarias batrachus* also possesses a liver specific LDH *C. gene.*

The relative mobilities of the LDH-B_4 and -A_4 isozymes in *Heteropneustes fossilis* and *Clarias batrachus*, are reversed. Further there are significant quantitative differences in the amounts of specific isozymes in homologous tissues of these two species and also in different tissues of a single species as documented in densitomeric tracings. The subunit composition of the LDH isozymes were confirmed by immunological procedures.

Keywords. Cypriniformes; lactate dehydrogenase; characterization; *Heteropneustes fossilis*; *Clarias batrachus.*

1. Introduction

Lactate dehydrogenase (EC 1.1.1.27) is a widely studied enzyme that is polymeric in many animals and is under multilocus control. The isozymes of LDH of most higher vertebrates are composed of two genetically and biochemically distinct polypeptides A and B. The apparent random union of these two polypeptides results in 5 possible tetrameric isozymes. These are the two homopolymers A_4 and B_4 and the 3 heteropolymers A_3B_1, A_2B_2 and A_1B_3 (Markert 1968). Although members of all vertebrate classes are known to possess the A and B genes for LDH, their individual isozyme repertories differ due to evolutionary changes in the structure of these two loci and to the characteristically different tissue specific expression of these loci in different species (Whitt *et al* 1973).

More complex isozyme patterns are known from several vertebrate groups. In addition to the A and B subunits, mammals and birds possess a third LDH subunit 'C' which is encoded by a third locus (Zinkham *et al* 1969; Holmes *et al* 1974). Unlike the two other LDH genes, the C locus of homeotherms exhibits an extremely restricted temporal and tissue specific expression, active only in primary spermatocytes (Hawtrey and Goldberg 1970). In teleost fishes also a third locus, the LDH C locus, has been established by genetic, biochemical and immunological studies which is usually expressed electrophoretically as either a highly anodal band in the neural tissue of many teleostean species or as a cathodal band in the liver of fishes of the orders cypriniformes and gadiformes.

The present study was undertaken to characterize the LDH isozyme patterns in the two cyprinids, *Heteropneustes fossilis* and *Clarias batrachus.*

2. Materials and methods

Fish used in this study were obtained live from local markets of Hyderabad, Andhra Pradesh. The heart, skeletal muscle, eye, brain, kidney, liver, intestine and gills were homogenized in equal volume of 0·01 M Tris HCl buffer pH 7·0. The homogenates were centrifuged for 30 min at 20,000 g and 4°C. After centrifugation the supernatants were subjected to polyacrylamide disc gel electrophoresis in 5·5 % gel formulated by Dietz *et al* (1971). Electrophoresis was performed at a constant current of 2·5 mA per tube for the first 5 min and then raised to 5 mA for the rest of the run for 60 min. Following electrophoresis LDH activity was visualized on the gels according to the method of Gade (1979). Control gels were stained with an identical solution lacking the substrate. Staining was performed in the dark at 37°C and terminated by washing and fixing the gel in 7·5 % acetic acid. The densitometer scans were performed on a Joyce's Loebel chromoscan and relative activity of isozymes was calculated from the area of each peak demonstrated by densitometry.

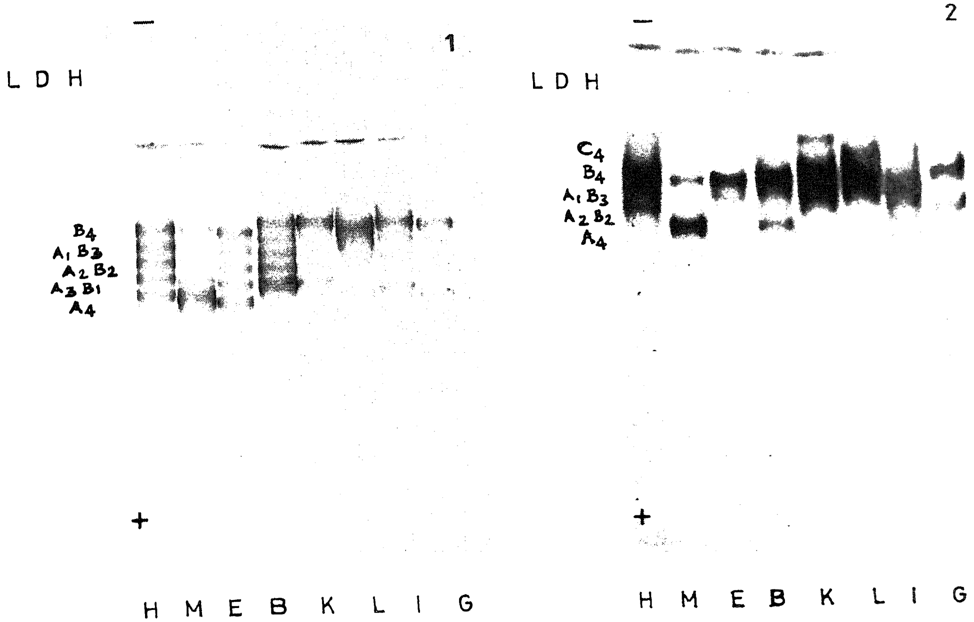
2.1 *Immunological characterization*

Antisera against purified LDH-A_4 and LDH-B_4 of *Alosa pseudoharengus* (Alewife) enzymes was provided by G S Whitt, University of Illinois, USA. For immunoprecipitation heart and muscle extracts of *H. fossilis* and *C. batrachus* were mixed with varying amounts of anti-A_4 and anti-B_4 in a constant volume (100 μl) and were incubated at 4°C for 2 hr and then centrifuged (Holmes and Markert 1969). The heart and muscle extracts of *H. fossilis* and *C. batrachus* were treated with 10, 15 and 20 μl of anti-A_4 anti-B_4 serum for obtaining enough inhibition. The resulting supernatants were subjected to electrophoresis and stained for LDH. The reduction or absence of specific isozyme bands on the gel after electrophoresis is an illustration of their immunochemical similarity.

3. Results

3.1 *Tissue specific distribution*

The tissue specific isozyme pattern of *H. fossilis* is given in figure 1. Five distinct bands of LDH activity were observed in all the tissues examined. The isozyme patterns for all the tissues examined are not qualitatively different in their number or electrophoretic mobility, although the relative intensities of the isozyme bands differ with the different tissues. Unlike the majority of vertebrates or even other teleosts *H. fossilis* exhibits a reversal of the relative electrophoretic mobility of the B_4 and A_4 isozymes. Since higher intensity is seen in the slow moving bands in the heart, the slow moving band in all other tissues from this fish is designated as LDH-B_4 isozyme. The higher intensity seen in muscle is in the fast moving band. Hence the fast moving band in all other tissues from this fish is designated as LDH-A_4 isozyme. Neither C_4 homopolymer nor heteropolymers containing C subunits were observed in either the liver or eye tissues. The percentage contributions of each isozyme calculated from the densitometer tracing of each tissue are given in table 1. The percentage contribution of LDH-A_4 in muscle is



Figures 1–2. Electrophoretic patterns of LDH isozymes of the heart (H), muscle (M), eye (E), brain (B), kidney (K), liver (L), intestine (I) and gills (G). **1.** *H. fossilis.* **2.** *C. batrachus.*

Table 1. Percentage composition of the LDH isozymes in *H. fossilis* and *C. batrachus.*

Species	Tissue	LDH-A_4	LDH-A_3B_1	LDH-A_2B_2	LDH-A_1B_2	LDH-B_4
H. fossilis	Heart	7·0	17·0	19·0	24·0	33·0
	Muscle	45·0	22·0	10·0	8·0	15·0
	Eye	15·0	18·0	17·0	20·0	30·0
	Brain	14·0	8·9	14·0	30·0	33·1
	Kidney	20·0	7·2	8·8	28·0	36·0
	Liver	19·0	4·0	3·0	20·0	54·0
	Intestine	17·0	16·0	17·0	18·0	32·0
	Gill	22·0	16·0	15·0	14·0	33·0
C. batrachus	Heart	25·0	—	18·0	22·0	35·0
	Muscle	44·5	—	20·9	16·0	18·6
	Eye	15·7	—	13·3	9·0	62·0
	Brain	20·0	—	18·0	22·0	40·0
	Kidney	16·0	—	27·0	22·0	35·0
	Liver	5·8	—	9·5	20·0	50·0
	Intestine	28·0	—	18·0	12·0	42·0
	Gill	15·5	—	30·0	22·0	32·5

more when compared to other tissues. Although the zymograms of liver and kidney of *H. fossilis* show 5 isozymes, LDH-A_2B_2 and A_3B_1 are conspicously less abundant than LDH-B_4, A_1B_3 and A_4. It can be seen from table 1 that most of the activity in heart extract is concentrated in the slowly migrating isozymes.

The LDH isozyme pattern of *C. batrachus* is represented in figure 2. This fish is a 4 isozyme fish possessing the A_4 and B_4 homopolymers and the heteropolymers A_2B_2 and A_1B_3. This fish shows higher band intensity in fast moving isozyme of muscle. Hence the fast moving isozyme in all tissues is denoted as LDH-A_4 and slow moving isozyme as LDH-B_4. As in other vertebrates, the A_4 and B_4 isozymes are evident in nearly every tissue examined. In this fish the electrophoretic mobility of LDH-B_4 and -A_4 is reversed as in the case of *H. fossilis*. The number of isozymes is the same for all the tissues except in liver and heart. Although this species lacks the characteristic highly anodal eye band seen in many teleosts, the products of the third LDH locus, form the predominant C_4 isozyme in the liver. It is obvious from the tissue specific pattern of *C. batrachus* that it is not restricted to liver tissue alone but is also synthesised in low levels in the heart tissue. Except for the muscle all the tissues exhibit an isozyme pattern consisting of an intensely staining LDH-B_4 isozyme. It is observed that the percentage of LDH-B_4 in eye is more when compared to the contribution of LDH-B_4 in other tissues. The percentage contribution of the cathodal isozyme C_4 in liver is 12. The C_4 isozyme in the heart is not scanned as it is very faint.

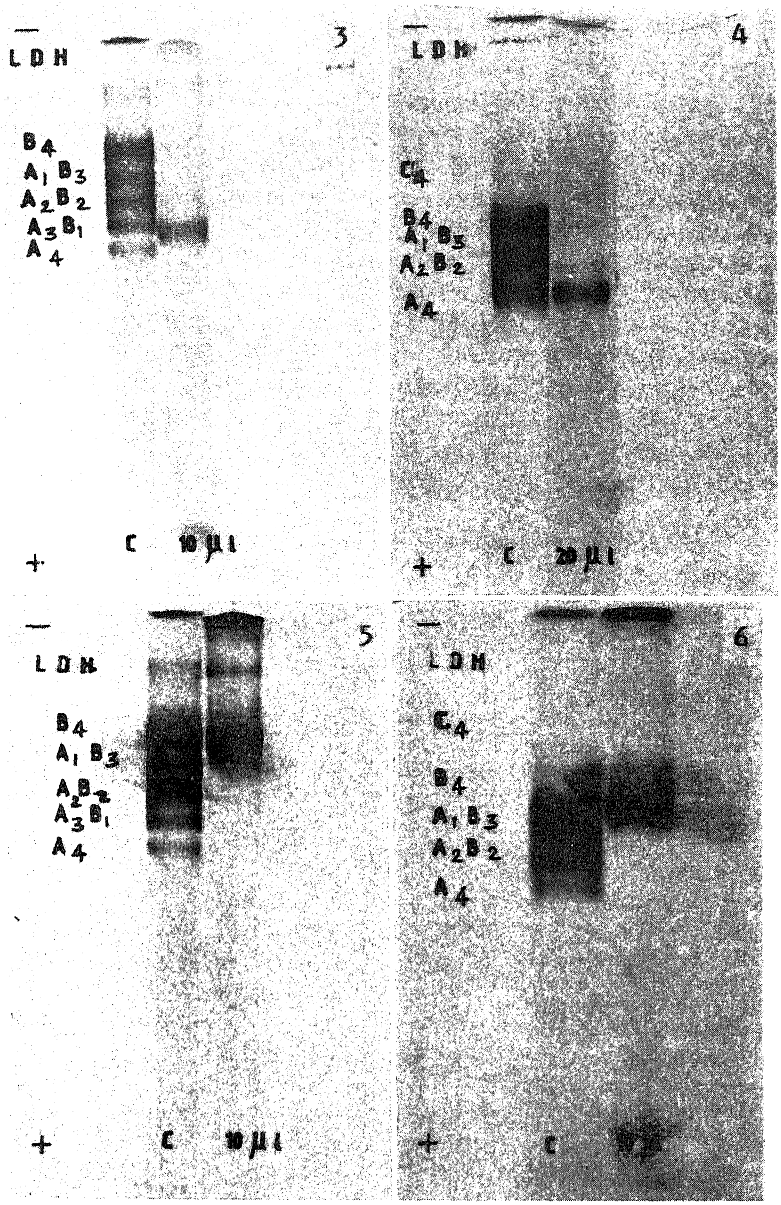
3.2 *Immunological characterization*

In *H. fossilis* and *C. batrachus* treatment of the heart tissue extract with anti-B_4 sera removed only LDH-B_4 isozyme (figures 3 and 4) while anti-A_4 sera removed only LDH-A_4 isozyme (figures 5 and 6). When the muscle tissue extracts from *H. fossilis* and *C. batrachus* were treated with anti-B_4 and anti-A_4 sera it was found that the anti-B_4 serum precipitates the B_4 isozymes (figures 7 and 8) while the anti-A_4 serum precipitates the A_4 isozymes (figures 9 and 10).

4. Discussion

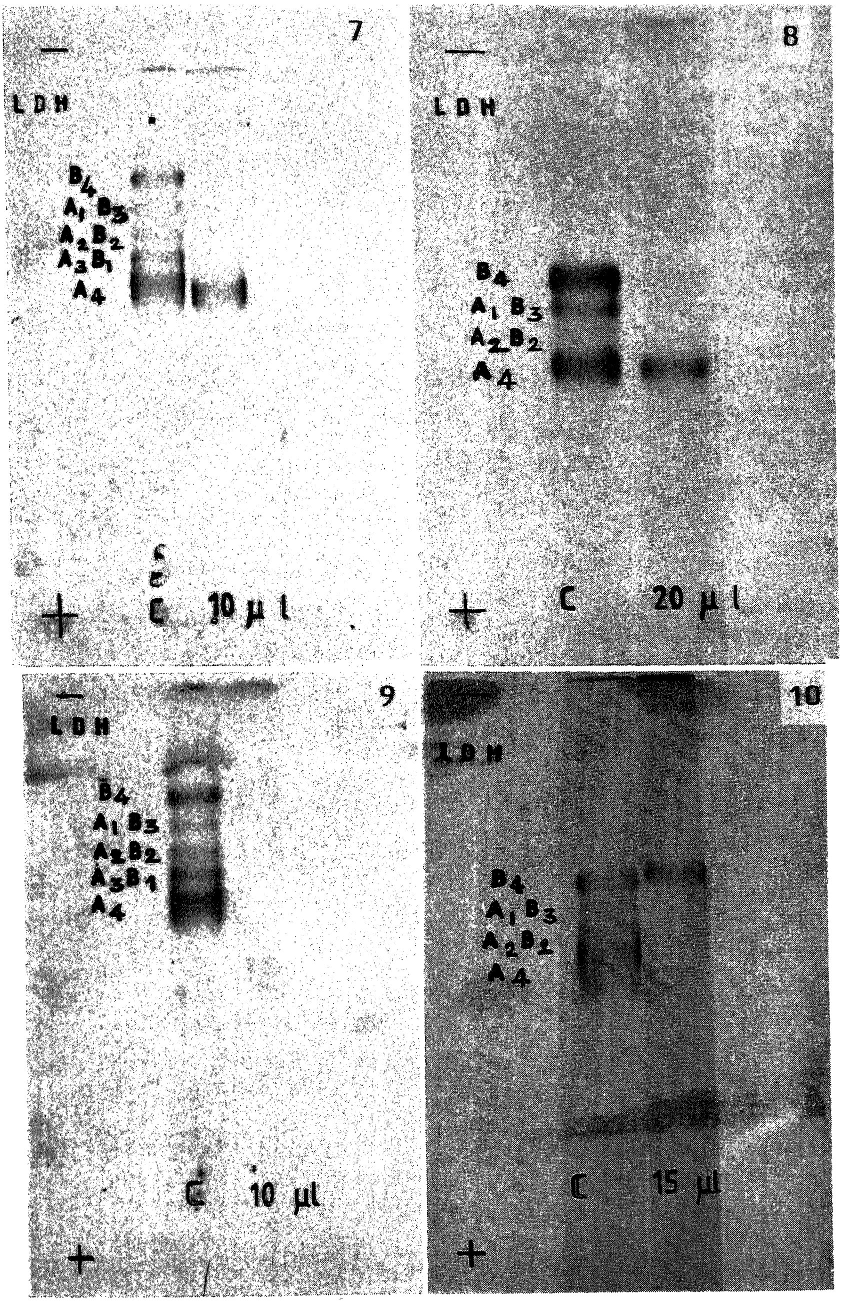
In vertebrates the LDH-B_4 isozyme is most abundant in heart, almost always carries a larger net negative charge over the pH range 7–9 than the LDH-A_4 isozyme which predominates in the skeletal muscle. However in fish the relative charges on these isozymes are more variable. The rapidly migrating isozyme (LDH-A_4) in *H. fossilis* and *C. batrachus* is more abundant in muscle. On the contrary most of the activity in the heart extracts of these fishes is concentrated in the slowly migrating isozyme (LDH-B_4). This is not an uncommon occurrence among fish as evidenced by the report that of 48 teleosts from 17 orders listed by Markert *et al* (1975), 11 showed greater anodal mobility for LDH-A_4 isozyme. The subunit composition of the isozymes and their homologies, initially assigned on the basis of their pattern of expression in all heart and muscle tissues were confirmed by immunological procedures. The A_4 anti serum reacted only with LDH-A_4 isozyme while the B_4 anti serum reacted with LDH-B_4 isozyme in both heart and muscle tissues of all the fishes irrespective of their electrophoretic mobility.

The appearance of 5 isozymes in the fish although similar to the situation in birds and mammals (Kaplan 1964) is not the rule in fishes. Of 30 species of fishes, Markert and Faulhaber (1965) found only 3, which produced all 5 possible A B tetramers. *H. fossilis* exhibits a 5 isozyme pattern and *C. batrachus* exhibits a 4 isozyme pattern. Finding of



Figures 3–6. Effect of anti-B$_4$ serum. **3.** Heart LDH isozymes *H. fossilis*. **4.** Heart LDH *isozymes of C. batrachus*. **5.** Heart LDH isozyme of *H. fossilis*. **6.** Heart LDH isozymes of *C. betrachus*.

such variable number of isozymes is not unusual among fishes (Cowan 1972; Whitt *et al* 1973; Champion and Whitt 1976). The 5 isozyme fish makes all the 5 tetramers, the 4 isozyme fish makes all the isozymes except for A_3B_1. The presence of only 4 isozymes is most likely the result of restricted subunit assembly (Whitt 1970a) or an instability of the asymmetrical heteropolymer A_3B_1 (Shaklee *et al* 1973). It is evident from the densitometric data of isozyme patterns, that there are significant quantitative differences in the amount of specific isozymes in different tissues of a given species.



Figures 7–10. Effect of anti-A_4 serum. **7.** Muscle LDH isozymes of *H. fossilis*. **8.** Muscle LDH isozymes of *C. batrachus*. **9.** Muscle LDH isozymes of *H. fossilis*. **10.** Muscle LDH isozymes of *C. batrachus*.

The isozyme pattern found in the liver tissues of these fishes is very striking. The LDH-B_4 isozyme in *H. fossilis* and *C. batrachus* is predominant in the liver tissues. In *Channa striata* and *Channa gachua* (Triveni 1980) and trout (Wuntch and Goldberg 1970) LDH-B_4 was found to be predominant in the liver tissue. In mammals LDH-A_4 predominates in the liver tissue which is assumed to be anaerobic. Eye, brain and kidney tissues of *H. fossilis* and *C. batrachus* have higher amounts of LDH-B_4 indicating aerobic pattern like corresponding, mammalian tissues. The LDH C gene was not detected in the eye tissues of either *H. fossilis* and *C. batrachus*. The absence of the C gene is not unique among fishes since a few other teleosts have also been reported to lack the C_4 isozyme; herring (Klose *et al* 1969), gold fish (Nakano and Whiteley 1965; Whitt 1970a) and silver hake (Uttar and Hodgins 1969). However, some families of fishes in a couple of advanced orders of teleosts exhibit a different pattern of function of the LDH C gene, particularly in the Cypriniformes and gadiformes. An important characteristic to note is that a third homopolymeric isozyme the C_4 isozyme is synthesized mainly in the liver of some fishes and possesses a cathodal mobility (Shaklee *et al* 1973). A similar pattern was also observed for *C. batrachus* where the liver tissue possesses the C_4 isozyme which is cathodal in migration. In *C. batrachus* the C_4 isozyme does not form heteropolymers with either A or B subunits. Shaklee *et al* (1973) also reported the lack of heteropolymeric isozymes containing A or C and B subunits in several species of cyprinids. They suggested that in these fishes the C subunit synthesis is probably temporarily or spatially isolated from the synthesis of A and B subunits, which are found to combine freely with each other. It was observed from the tissue specific pattern of *C. batrachus* that the C_4 isozyme although predominates in the liver is not restricted to liver tissue alone but is also synthesised in low level in heart tissue. Analysis of the LDH isozyme patterns of over 100 species of teleost fishes representing some 61 families and 17 orders by Markert *et al* (1975) revealed that all species examined possess only either a liver or eye band of LDH.

Both the eye (Whitt 1970a, b) and the liver form (Sensabaugh and Kaplan 1972) of the C_4 tetramers differ significantly from the other two homopolymers. Therefore the distinctive tissue distribution and biochemical properties of the C subunit suggests that this particular gene product has been specifically tailored to occupy a more specialized metabolic niche. Because of its association with the photoreceptor cells in the retina of many teleosts the eye band (C_4) has been hypothesized to play a role in the visual physiology of some fishes (Whitt *et al* 1973). Similarly it has been suggested that the liver band (C_4) of cyprinids may be uniquely adapted to the peculiar biochemical characteristics of the liver of these fish or be involved in unique control mechanisms.

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Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats

B B KALIWAL, R NAZEER AHAMED and M APPASWAMY RAO
Department of Zoology, Karnatak University, Dharwad 580 003, India

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Abstract. In the present study the effect of petroleum ether extract of carrot seeds and progesterone on implantation have been investigated. 0·6 ml/100 g body weight of carrot seed extract was administered subcutaneously from days 1–7 of pregnancy and laparatomized on day 8. No implantation sites were observed. Thereafter graded doses of progesterone 2, 4 or 8 mg/100 g body weight was administered upto day 15 and autopsied on day 16. Two mg of progesterone did not show any effect. However, 4 mg of progesterone treated rats had smaller implantation sites compared to the controls whereas, 8 mg of progesterone was most effective with the result that in almost all the rats, delayed implantation was observed.

Keywords. Implantation; carrot seed extract; progesterone; albino rats.

1. Introduction

The use of plant extracts to induce changes in human fertility has been practised for centuries in many countries (Casey 1960). Many pasture plants exhibit antifertility activity, probably due to phytoestrogens in grazing live stock and domestic animals (Farnsworth *et al* 1975; Briggs and Christie 1977). It has been noted that any imbalance in the progesterone-estrogen ratio inhibits implantation in rats (Cochrane and Meyer 1957). Administration of antiestrogenic compounds or high dose of estrogens inhibits or delays implantations in rats (Cochrane and Meyer 1957; Chang and Yanagimachi 1965; Prasad and Kalra 1967). Many plant material or seed extracts are known to have estrogenic or antifertility activities (Kholkute and Udupa 1976; Sharma *et al* 1976). Alcoholic, aqueous or petroleum ether extract of carrot seeds prevent implantations in rats and mice, probably, due to its weak estrogenic activity as evidenced by luteotrophic uterine response (Garg and Garg 1971; Sharma *et al* 1976; Kaliwal and Rao 1977). The present study is designed to know the effect of progesterone in the antiimplantation activity of the carrot seed extract in the albino rats.

2. Materials and methods

Powdered carrot seed extract was Soxhleted with petroleum ether as described earlier (Kaliwal and Rao 1977, 1979). Colony bred, adult, nulliparous female albino rats of Holtzman strain, 75–85 day old with a body weight ranging from 130–170 g were used. After checking the rats for 3 regular estrous cycles, they were caged with males for mating. Mating was confirmed by the presence of sperms in the vaginal smears on the following morning designated as day 1 of pregnancy. Laparotomy was performed on day 8 of pregnancy by mid ventral incision under mild ether anaesthesia in semi-sterile condition.

In normal mated rats, 0·6 ml/100 g body weight of carrot seed extract was administered subcutaneously from days 1–7 of pregnancy, laparotomized on day 8 to note the number of implantations and autopsied on day 16. Thereafter in another group, 2, 4 or 8 mg of progesterone was administered till day 15. Suitable controls were maintained.

All the rats were autopsied on day 16 by cervical dislocation. Ovaries, uteri and adrenals were dissected out free from adherent tissues and weighed. Ovaries were processed for histological observations.

All the experimental rats were maintained in individual cages with Hindustan Lever rat pellets and water *ad libitum* at a room temperature of 27° ± 1°C with a lighting schedule of 14 hr of day light. The results were analysed statistically using Student's '*t*' test.

3. Results

3.1 *Delayed implantation* (*table* 1)

Controls exhibited implantations on day 8 and small embryos on day 16. When carrot seed extract was given from days 1–7, there were no implantation sites on day 8. The second group treated with carrot seed extract and graded doses of progesterone from days 8–15 gave the following results: 2 mg of progesterone was ineffective resulting in 100 % preimplantation loss. However, with 4 mg or 8 mg of progesterone almost all the rats had implantations on day 16. However, the total number and size of implantations was smaller in rats receiving 4 mg of progesterone.

3.2 *Body and organ weights* (*table* 2)

3.2.1 *Body weights*: Controls recorded a gradual and considerable increase in the body weight whereas, carrot seed extract treated rats showed a decrease. There was very little increase in the body weight in the carrot seed extract treated rats receiving progesterone.

3.2.2 *Ovarian weights*: Ovaries of controls weighed heavy and had well developed corpora lutea. In carrot seed extract treated rats with or without progesterone treatment, there was significant reduction in the ovarian weight ($P < 0·001$). The corpora lutea in these animals were small.

Uterine and adrenal weights: In controls, the uterus was heavy due to the developing foetuses. In the carrot seed extract treated rats, the uterine weight was considerably reduced due to inhibition of implantation and resembled that of nonpregnant rats. The uterus of 2 mg progesterone treated rats was approximately similar with that of nonpregnant rats whereas, in those receiving 4 mg of progesterone, there was slight increase in the weight as the number and size of the implantation sites was smaller. But 8 mg of progesterone treated rats exhibited larger implantation sites, resulting in the increase in uterine weight.

The adrenal weights in all the treated groups exhibited increase when compared to the olive oil treated controls probably, due to stress.

Table 1. Delayed implantation by progesterone in carrot seed extract treated rats.

		No. of rats					Implantation at				
			Pregnant at		Corpora lutea		Laparotomy		Autopsy		
Group	Treatment	Mated	Laparotomy	Autopsy	Total	Mean M ±S.E	Total	Mean M ±S.E	Total	Mean M ±S.E	Preimplantation loss at autopsy (%)
A	Olive oil (Control)	5	5	5	41	8·20 ±0·58	41	8·20 ±0·51	40	8·00 ±0·45	2.43
B	Carrot seed extract only	5	0	0	40	8·00 ±0·32	0	0·0[a]	0	0·0	100·0
	Days 1–7 *Days 8–15*										
C	Carrot seed extract + 2 mg progesterone	5	0	0	42	8·40 ±1·08	0	0·0	0	0·0	100·0
D	" +4 mg "	5	0	5	44	8·80 ±0·58	0	0·0	42	8·40 ±0·40	4·54
E	" +8 mg "	5	0	5	43	8·60 ±1·08	0	0·0	38	7·60 ±0·81	11·62

Treatments:	Dose/100 g body wt/day	
Carrot seed extract from days 1–7 of pregnancy		
Laparotomy on day 8	Olive oil	–0·6 ml (Days 1–15)
Progesterone— days 8–15	Carrot seed extract	–0·6 ml
Autopsy—Day 16	Progesterone	–2–8 mg

$$\%\ \text{Preimplantation loss} = \frac{\text{Total number of corpora lutea} - \text{Total number of implantations}}{\text{Total number of corpora lutea}} \times 100$$

M ±S. E = Arithmetic mean ±Standard error.

[a] $P < 0{\cdot}001$

Table 2. Effect of carrot seed extract and progesterone on body and organ weights in rats.

Group	Treatment	Increase in body weight (%) (g)	Organ weights (mg)/100 g body Wt. M ±S.E		
			Ovaries	Uterus	Adrenals
A	Olive oil (Control)	(5) 45·05	39·10 ± 1·02	5507·82 ± 608·20	26·49 ± 0·86
B	Carrot seed extract only	(5) 11·27	24·72 ± 1·65^{c}	149·19 ± 37·77	40·36 ± 3·44
	Days 1–7 *Days* 8–15				
C	Carrot seed extract +2 mg progesterone	(5) 2·87	27·15 ± 1·92	105·59 ± 24·26^{c}	37·61 ± 3·29^{a}
D	" +4 mg "	(5) 6·18	25·32 ± 1·56^{c}	297·95 ± 39·92^{c}	36·26 ± 1·66^{b}
E	" +8 mg "	(5) 6·94	26·08 ± 0·76^{c}	968·27 ± 289·30^{c}	43·75 ± 10·08^{b}

Treatments:
Carrot seed extract from days 1–7 of pregnancy
Laparotomy on day 8
Progesterone from days 8–15
Autopsy—Day 16

Dose/100 g body wt/day
Olive oil – 0·6 ml (Days (1–15)
Carrot seed extract – 0·6 ml
Progesterone – 2–8 mg

Number in parantheses denotes the number of rats.
M ±S. E. = Arithmetic mean ±Standard error.

$^{a}P < 0·05$; $^{b}P < 0·01$; $^{c}P < 0·001$.

4. Discussion

Implantation is a critical phase in the ontogeny of eutherian mammals, involving regulated co-ordinated changes in the uterus, uterine fluids and the blastocyst in sequential order by synergistic action of estrogen and progesterone (Prasad and Kalra 1967). Inhibition of implantation by chemicals and hormones may be due to their blastotoxic effect, expulsion of blastocyst, antihistaminic reaction, delayed transportation of the blastocyst into the uterus, imbalance in the progesterone-estrogen ratio or cessation of estrogen surge on day 3 or 4 of pregnancy in rats (Cochrane and Meyer 1957; Shelesnyak *et al* 1963; Chang and Yanagimachi 1965; Prasad *et al* 1965).

In the present investigation, administration of petroleum ether extract of carrot seeds, inhibited implantation in almost all rats. This may be due to the imbalance in progesterone-estrogen ratio of 4000 : 1 as stated by Cochrane and Meyer (1957) in their delayed implantation experiments in rats. If exogenous progesterone was administered from days 8–15 to the carrot seed extract treated rats, all of them retained the capacity to implant and exhibited implantations on day 16. The size of the implantation sites was depended on the dose of progesterone. This may indicate that carrot seed extract may neither be blastotoxic nor it might have caused expulsion of blastocyst as in the case of estrogens (Burdrick *et al* 1937; Morris *et al* 1967). Any imbalance in the hormonal ratio causes delay or inhibits implantation (Nutting and Meyer 1963; Prasad *et al* 1965). Therefore, the inhibition or delay of implantation by carrot seed extract might be due to an imbalance in the progesterone-estrogen ratio which could be reversed by the administration of exogenous progesterone. However, further investigation is necessary to elucidate the mechanism of action of carrot seed extract on the inhibition or delay in implantation in albino rats.

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Some observations on the biology of periwinkle *Littorina scabra scabra* (Linne 1758)

S MARUTHAMUTHU and R KASINATHAN

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, India

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Abstract. Reproductive behaviour in *Littorina scabra* (Linne 1758) was studied from Porto Novo jetty area and observations were made both in natural habitat and laboratory condition. Copulation was observed throughout the year whenever moisture was abundant. It lasts usually 30–50 min and pairs remain together for several hours after copulation has been completed. Copulation was correlated with lunar phase. Breeding in *Littorina scabra* was observed throughout the year and spawning occurs during high tide of the newmoon and fullmoon. It is concluded that the breeding cycle completes within two weeks. There is no correlation between the number of offspring and the size of the individual. The first indication of sex cells appear in males and females at the size of 9·02 mm and 8·7 mm respectively. This result indicates that male matures before females. Sex-ratio of *Littorina scabra* was 1:1·44 which showed fluctuation when considered month-wise. Mean shell height of male and female were equal. The development was normal in the salinity ranges of 28–30% and at the temperature of 30°C. The veliger emerged out of the egg at 96 hr after the cleavage has started.

Keywords. *Littorina scabra scabra*; Chi-square test; F-test.

1. Introduction

Littorina scabra occurs in appreciable quantities at different places in the Indo-Pacific region (Rose Water 1970). Littorinids especially *L. littorea* are of large size and they are of nutritional value in certain countries. Periwinkles (*L. littorea*) are gathered in many places and shipped to markets in certain western countries.

Studies on littorinids are few from Indian waters. The preliminary report available on the life history of *L. scabra* from Indian waters is that of Sewell (1924).

Since there is no information available about the spawning season, sex-ratio, the size at first maturity and early larval development of the periwinkle of *L. scabra* from Vellar Estuary, the present investigation was undertaken.

2. Materials and methods

Monthly samples from Porto Novo jetty area were used to determine the sex-ratio by examining live snails for the presence or absence of penis (Struhsaker 1966). Chi-square test was pertained to test the homogeneity within the male and female of *L. scabra*.

Smear examinations of gonad helped to determine the size at which sexual maturity was attained.

The percentage of *L. scabra* pairing during different phases of moon was determined from 21 pillars of Porto Novo jetty area every morning in September from 1st to 22nd, 1983. Copulation was observed in field and laboratory conditions.

The portions of gonad from monthly samples were fixed in Bouin's fixative, embedded in paraffin wax and sections were cut at 9μm thickness, stained with Delafield haematoxylin and eosin. The spawning periods were ascertained by smear examinations and histological methods as done by Alagarswami (1966) and Nagabhushanam and Mane (1975).

Breeding period of *L. scabra* was determined by observing the presence of eggs or embryo in the mantle cavity of the living animal (Lenderking 1954; Gallagher and Reid 1974) which appears as a cream coloured or yellow mass. Animals collected to study the spawn release 1-2 days before full or newmoon were isolated and placed in small bowls containing estuarine water (salinity 30‰) in a depth of 10 cm at room temperature (27–31°C). The time of the day when the females spawned was checked to ascertain any tidal rhythm. Veligers or eggs were counted from the egg sheet to note the number of eggs spawned.

Fresh egg masses were removed from the mantle cavity of the animal and cultured in fingerbowls containing estuarine water at a temperature of 27°–30°C and a salinity range of 28–30‰. The fingerbowls were frequently changed, the development was followed and camera lucida drawings were made. Different stages were fixed in 5% formalin and 70% alcohol.

3. Results

3.1 *Sex ratio*

Of 1121 individuals of *L. scabra* examined to determine the sex ratio, 662 (59·05 %) were females and 459 (40·95 %) were males. Based on the total number of specimens observed

Table 1. Number and percentage of male and female of *L. scabra* in various months of the year 1982–1983.

Month	Sample size	Males		Females	
		Number	Percentage	Number	Percentage
December 1982	91	30	32·97	61	67·03
January 1983	100	41	41·00	59	59·00
February	39	34	38·20	55	61·80
March	93	42	45·16	51	54·84
April	96	43	44·79	53	55·21
May	87	27	31·03	60	68·97
June	99	31	31·31	68	68·69
July	91	37	40·66	54	59·34
August	94	35	37·23	59	62·77
September	91	48	52·75	43	47·25
October	90	35	38·89	55	61·11
November	100	56	56·00	44	44·00
	1121	459	40·95	662	59·05

in this study the male: female ratio was found to be 1:1·44, indicating the preponderance of females over the males (tables 1 and 2).

The sex ratio of the population was calculated monthwise for the period of December 1982 to November 1983 and Chi-square with yates correction for continuity was applied on monthly random samples to test whether the population follows 1:1 ratio. When considered monthwise, the males were found to be dominant during September and November only.

The sex ratio of different size group is represented in table 3. The females were dominant in all groups except 9·1–9·7 and 13·3–13·9 mm.

Table 2. Sex ratio and Chi-square values for *L. scabra* in different months.

Months	Male	:	Female	Chi-square values	Remarks $P = 0{\cdot}05$
December 1982	1	:	2·03	9·89	Significant
January 1983	1	:	2·89	2·89	Insignificant
February	1	:	1·62	4·49	Significant
March	1	:	1·21	0·69	Insignificant
April	1	:	1·23	0·84	Insignificant
May	1	:	1·22	11·77	Significant
June	1	:	2·19	13·09	Significant
July	1	:	1·46	2·81	Insignificant
August	1	:	1·69	5·63	Significant
September	1	:	0·90	0·18	Insignificant
October	1	:	1·59	4·01	Significant
November	1	:	0·79	1·21	Insignificant
	1	:	1·44	36·40	Significant

Table 3. Sex ratio and Chi-square value for *L. scabra* in different size groups.

Size groups (mm)	Male (n)	Female (n)	Male	:	Female	Chi-square value	Remarks $P = 0{\cdot}05$
03·5–04·1	0	5		–		03·20	Insignificant
04·2–04·8	0	2		–		00·50	Insignificant
04·9–05·5	4	5	1	:	1·25	0	Insignificant
05·6–06·2	14	27	1	:	2·93	03·51	Insignificant
06·3–06·9	13	32	1	:	2·46	07·20	Significant
07·0–07·6	29	63	1	:	2·17	11·84	Significant
07·7–08·3	52	71	1	:	1·37	02·63	Insignificant
08·4–09·0	61	90	1	:	1·48	05·19	Significant
09·1–09·7	85	70	1	:	0·82	01·26	Insignificant
09·8–10.4	57	66	1	:	1·16	00·52	Insignificant
10·5–11.1	52	87	1	:	1·67	08·32	Significant
11·2–11·8	39	40	1	:	1·03	0	Insignificant
11·9–12·5	27	42	1	:	1·56	02·84	Insignificant
12·6–13·2	8	15	1	:	1·88	01·57	Insignificant
13·3–13·9	11	10	1	:	0·91	0	Insignificant
14·0–14·6	6	21	1	:	3·5	07·26	Significant
14·7–15·3	1	13	1	:	13·00	08·64	Significant
15·4–16·0	0	02		–		00·50	Insignificant
16·1–16·7	0	01		–		0	Insignificant

3.2 *Size differences in sex (sexual dimorphism) and size at sexual maturity*

The mean shell height was 9·59 mm, when considered sex-wise, the male has mean shell height of 9·6 mm while that of female was 9·58 mm. However, the size groups 14·7–15·3; 15·4–16·0 and 16·1–16·7 were available in females only with one exception in the size group of 14·7–15·3 mm, a male.

Statistically F-test (table 4) was applied to authenticate the differences in mean shell height of male and female individually.

Smear examination of gonads, reveals that the first indication of sex cells appeared in females in the size of 9·02 mm and in males in the size of 8·7 mm.

3.3 *Copulation*

Table 5 shows the numbers and percentage of snails pairing at different days. The pairing of snails reached a peak 5–6 days before the fullmoon and newmoon days. No pairing was observed on 22nd September 1983 (fullmoon) and 1·66% pairing was noticed on 6th September 1983 (newmoon). 20·90% pairing was observed on 2nd

Table 4. ANOVA table.

Source of variation	SS	DF	MS
Total	3394·519	661	
Groups	571·952	11	51·9956
Error	2822·567	650	4·3424

$$F = \frac{\text{groups Ms}}{\text{error Ms}} = 11{\cdot}9739$$

Highly significant Hi. $P < {\cdot}001$

Source of variation	SS	DF	MS
Total	1395·620	458	
Groups	223·357	11	20·3052
Error	1172·262	447	2·6225

$$F = \frac{\text{groups Ms}}{\text{error Ms}} = 7{\cdot}7427$$

Highly significant $P < 0{\cdot}001$

Source of variation	SS	DF	MS
Total	5068·88	1120	
Groups	0·16	1	0·16
Error	5068·72	1119	4·6191

$F = 0{\cdot}0346$

Not significant $P > 0{\cdot}05$

Table 5. Lunar periodicity of mating in *L. scabra*. The number of snails represents the largest number pairing on the date shown. Total population was counted every day; percentages are based on this number.

Date Sep. 1983	Total population	No. of individual snails pairing	Snails pairing (%)	Moon phase
September 1st	195	39	20·00	
2	201	42	20·90	
3	191	19	9·45	
4	185	6	3·24	
5	179	4	2·23	
6	180	3	1·66	Newmoon
7	No pairs found	–	–	
8	–	–	–	
9	–	–	–	
10	201	2	1·00	
11	186	6	3·23	
12	182	4	2·20	
13	185	6	3·24	
14	201	15	7·45	
15	179	22	12·29	
16	175	42	24·00	
17	182	43	23·05	
18	186	15	08·06	
19	182	3	01·65	
20	180	2	01·01	
21	No pairs found	–	–	
22	No pairs found	–	–	Fullmoon

September and 24% on 16th September 1983. Thus it would appear that mating behaviour exhibits a bilunar periodicity.

During copulation, the male fastens to the right side of the female with its foot while the female is also firmly attached to the substrate with its foot. The penis is inserted into the mantle cavity of the female through the right side of the shell aperture. The distal portion of the penis is inserted into the opening of the oviduct. During release of sperm, the basal portion serves to hold the penis in place. The duration of copulation in *L. scabra* is between 30–50 min. Copulation normally occurs in wet areas of the habitat. Pairs remain together for several hours even after copulation.

3.4 *Breeding*

From the smear examinations, histological sections of gonads and incidence of eggs or embryo in the mantle cavity (from December 1982 to November 1983) it would appear that *L. scabra* breeds throughout the year.

Breeding by *L. scabra* follows bilunar and tidal rhythms. The females release the veliger larval at the time of the full and the newmoon and this continued for approximately 4–5 days thereafter. Spawning seems to be correlated directly with lunar periodicity and breeding cycle was completed within 2 weeks. During spawning, the female moved to the bottom of the container and crawled for about 90 min, the release of veligers coinciding with the highest tide indicates a tidal rhythm.

While smaller (9·5 mm) snails released about 72,999 larvae, the larger ones (15 mm) released as many as 1, 02, 121 larvae per spawning. The large snails did not always release the maximum number of veligers (table 6).

Female lays large number of eggs after copulation which are fertilized as they pass through the oviduct, to the albumen gland and each egg after being covered by a hyaline egg case passes out through the female opening into the mantle cavity. The eggs collected from the mantle cavity are pale yellow with a diameter of 55 and 60 μm. Total diameter of eggs ranged between 65–72 μm with the hyaline covering. Length of the individual egg masses varied between 9·5 and 10 mm, the width being 4·5 mm.

3.5 *Early development* (*figure* 1)

Developmental stages were examined in fresh egg masses collected from the mantle cavity. The earliest stage noticed was one-celled stage prior to release of the polar bodies. A small clear area was observed at the one-celled stage, the rest contained dense yolk granules. Just prior to polar body extrusion a change in the configuration of the clear area was observed. Polar body was formed at the vegetal pole, opposite to the clear area. After the polar body formation, the clear area vanished and the egg appeared uniformly dense throughout.

The first cleavage was formed at about 2 hours after the polar body extrusion, resulting in 2 blastomeres. At 2–2¾ hr 70 % of eggs were in the 4-cell stage. Each of the 4 blastomeres appeared uniform in the distribution of yolk granules. The third cleavage was observed at 6–9 hr, resulting in 4 small micromeres and 4 large yolky macromeres. Seventy percent of eggs completed their third cleavage in 7 hr. The 12 cell stage was formed by a vertical division of 4 upper micromeres into 8, while the macromeres lingered as before. The embryo, then appeared as a mass of blastomeres, the number of segments could not be reckoned after the 32 cells. Owing to the subsequent cleavages, the micromeres formed a cap of clear cells on the yolky macromeres. Gastrulation occurred at 15 hr and 75 % of the embryos were covered by a cap of clear cells at 25 hr. There was an inner mass of yolky cells surrounded by a layer of clear cells. Gastrulation was completed at 30–32 hr and the embryo began to rotate. At 41 hr the embryo assumed a triangular shape representing the trochophore with cilia and the rotation

Table 6. Size of females and numbers of offspring produced.

Size	Number checked	Number of offspring
15·0	1	1,02,121
14·4	2	99,120
14·5	2	95,152
12·6	3	85,163
11·0	4	75,250
10·5	1	99,121
10·0	6	88,077
09·5	3	97,290
09·0	6	72,999

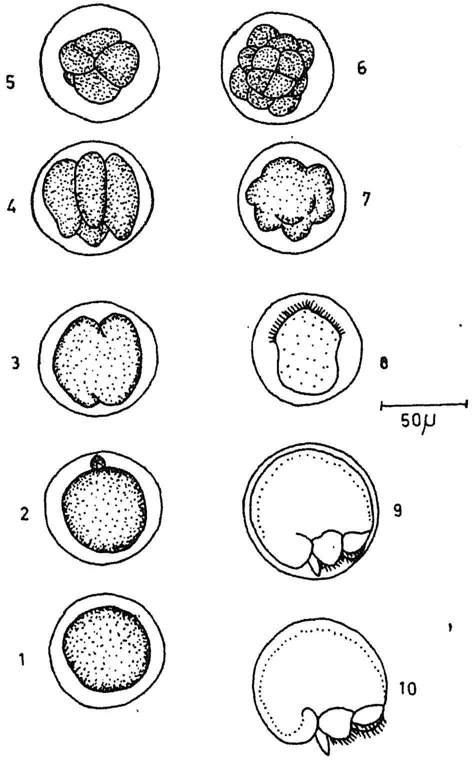

Figure 1. Larval development of *L. scabra*. **1.** Egg. **2.** Polar body formation. **3.** 2 Celled stage. **4.** 4 Celled stage. **5.** 8 Celled stage. **6.** 12 Celled stage. **7.** Multicelled stage. **8.** Trochophore stage. **9.** Veliger. **10.** Developed veliger.

was clearly observed within the egg covering. At 63 hr the triangular shape was more pronounced and the rotations were more rapid (15–18 rotations in 1 min). By 69 hr the early veliger stage was discernible. At 75 hr, the operculum and the ciliated foot were observed. The diameter of egg covering increased from 72–94 μm. Forty five per cent of veligers were observed at 84 hr in the fingerbowls and 100% at 96–99 hr.

The operculum of larvae was fastened to the tiny larval foot, which helps the animal to crawl on the substratum. The veligers settled on the bottom of the container. The foot was ciliated dorsally and when disturbed the velar lobes and foot were both withdrawn into the larval shell and the operculum could completely close the mouth of the shell. Velar lobes were not equal in size with their marginal row of cilia form effective organs for locomotion, while in locomotion the velor lobes were fully extended. The larvae which were 89 μm in length at the initial stages of observation reached the maximum length of 106·5 μm by 9th day, and after the 12th day the larvae were all dead owing to lack of proper food.

4. Discussion

The differences in size between the sexes are found to be significant and the mean shell height is almost equal in both sexes. The mean shell height of animals in every month is

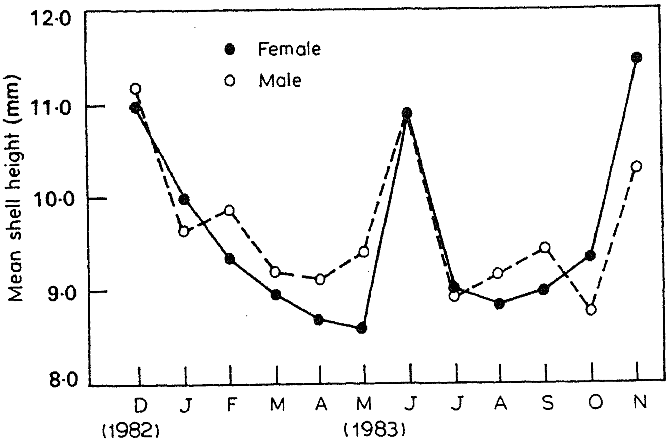

Figure 2. Mean shell height of male and female in different months of *L. scabra*

not the same in male and female (figure 2). Gallagher and Reid (1974) also recorded significant value in *L. scabra angulifera* and insignificant value was observed in *L. irrorata*. Sacchi (1980) recorded that the females were often larger than males in *L. saxatilis*.

Present investigation revealed that sexual maturity is being attained at 9·02 mm in females and 8·7 mm in males. This observation indicates that male matures before the females. It shows that reproductive strategy adopted by males resulting from competition for mates. This conclusion agrees well with that of Hannaford Ellis (1983).

The sex ratio was 1:1·44 indicating a preponderance of females (table 2). Monthwise sex ratio of *L. scabra* fluctuated and significant values were observed during December, February, May, June, August and October and insignificant values were observed during January, March, April, July, September and November. Significant values were observed in the following size groups 6·3–6·9; 7·0–7·6; 8·4–9·0; 10·5–11·1; 14·0–14·6; 14·7–15·3. Berry and Chew (1973) indicated equal proportions of males and females in the population of *L. melanostoma*. The differences seems to be either related to the species or to environmental influences. The greater number of females in the estuarine population of *L. scabra* is similar to that reported for *L. neritoides* (Daguzan 1976) and *L. saxatilis* (Sacchi 1980).

Copulation occurs throughout the year following lunar rhythms. Animals in aquaria and field did not copulate in dry condition and this observation agrees with that of Lenderking (1954), Gibson (1964) and Struhsaker (1966). Lunar periodicity of copulatory behaviour of *L. scabra* is similar to that of *L. scabra angulifera* (= *L. angulifera*) (Gallagher and Reid 1974). There was apparently to size selection in the copulatory behaviour of *L. scabra*.

Breeding in *L. scabra* occurs throughout the year in Porto Novo water during the newmoon and the fullmoon at high tide. The present result agrees well with that of Sewell (1924), Lebour (1945), Struhsaker (1966) and Heller (1975) (*L. rudis*).

The present findings did not reveal any correlation between the number of offspring produced and the size of the individual, thus supporting the view of Lenderking (1954). The data indicate that *L. scabra* produces more offsprings than other littorinids species reported (Borkowski 1970; Hughes and Roberts 1980).

The eggs are retained in the mantle cavity which functions like a brood pouch and the development upto free swimming veliger stage takes place here. The animal is Ovoviviparous (Rosewater 1970). Eggs of *L. scabra* differ from those of *L. littorea* (Goodwin 1979) and *L. melanostoma* (Berry and Chew 1973) being pale yellow spherical and encircled by a hyaline slightly avoid case as reported by earlier workers (Struhsaker 1966; Lenderking 1954).

According to Gallagher and Reid (1974), the development of *L. scabra angulifera* took approximately 4 days. The time taken for development from egg laying to hatching in *L. littorea* is about 12–14 days (Fish 1979) while in the present study the development of *L. scabra* was found to be in 3½–4 days.

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Table 1. World distribution of genera of Ariidae.

	Ocean and FAO fishing area			
	Eastern Indian 57	Western Indian 51	Western Central Pacific 71	Western Central Atlantic 31
Genus	No. of species	No. of species	No. of species	No. of species
Ariopsis	—	—	—	×3
Bagre	—	—	— —	×2
Cathrops	—	—	—	×9
Potamarius	—	—	—	×2
Gaieichthys	—	—	—	×3
Doiichthys	× 1	—	× 1	—
Hemipimelodus	×11	—	×11	—
Ketengus	× 1	—	× 1	—
Nedystoma	× 1	—	× 1	—
Tetranesodon	× 1	—	× 1	—
Batrachocephalus	× 1	× 1	× 1	—
Osteogeneiosus	× 1	× 1	× 1	—
Arius	×58	× 18	×59	×9
Ancharius	—	×	—	—

(1886) recorded *Arius* species from the Siwalik hills, India. Newton (1889) described fossils of *A. bartoni* from Eocene beds of Barton, England. Woodward (1901) recorded *A. crassus* from the Middle Eocene of Bracklesham, England and *A. iheringi* from the Tertiary lignite of Brazil. Rao (1956) recorded *A. kutchensis* from the Lower Eocene of Kutch, Gujarat state, India. More recently Khare (1975) recorded *A. sahnii* and *Arius* sp. from Middle Eocene Maroon argilaceous sandstones of Subathu, formation exposed near Beragua Coal Field, Jammu and Kashmir. From these evidences, it appears that the Ariidae seems to have existed as early as Eocene.

Considering the fossil history of other siluroid families, it has already been stated that the Siluroids as a group can be traced only from Cretaceous era. The Bagridae are known as fossils from Pliocene and Pleiostocene.

3. Phylogeny of Ariidae

Gosline (1975) considered the intensive and extensive investigation of particular structure or structural systems as a possible approach to the unravelling of siluroid phylogeny. He considered some units like the caudal skeleton, pelvic girdle, pectoral girdle, Weberian apparatus and associated structures and otoliths as helpful tools. Chardon (1968) in fact utilised some of these in building up his classification of the Siluroids. However, not much information is available in respect of Ariidae. Regan (1911), Eigenmann (1921) and Berg (1940) considered Ariidae as the most primitive next to Diplomystidae, whereas Bertin and Arambourg (1958) thought Bagridae as the most primitive member. Bhimachar (1933) on the basis of his studies on the osteocranium of *Arius* concluded that it is a more specialised member than other families. Gosline (1944) and Tilak (1965) also came to similar conclusion. Srinivasachar (1980) adduced evidence from chondrocranial studies of some siluroid genera, that *Arius* has branched quite early in a different direction from a primitive *Plotosus* like ancestor. Chardon (1968) in his work, opined that the generalised stock of Siluroidei gave rise to many suborders probably independently. The suborder Bagroidei was considered as having evolved as a result of adaptive radiation. Ariidae was thought to have arisen from a Plotosid ancestor, in support of which it may be stated that Plotosidae are the only Catfishes to share with Ariidae an enormous utriculus.

4. Interrelationship of Indian genera

In the light of studies on the Indian genera of the family Ariidae certain data are presented towards this problem.

Ariidae is represented by 22 species under 5 genera in Indian waters: *Arius* (18), *Osteogeneiosus* (1), *Hemipimelodus* (1), *Ketengus* (1), and *Batrachocephalus* (1). Of these *Hemipimelodus* is almost similar to *Arius* except in the fact that it has an edentate palate and as such is not considered. Many workers like Misra (1976) and others have misidentified some species of *Arius* as referable to *Hemipimelodus* and *vice versa*.

Of the 4 Indian genera excluding *Hemipimelodus* a comparison of certain osteological and a few other morphological characters clearly indicate that *Arius* is the most specialised of all. The compact cranium, well developed ethmoid bones and epioccipital lamellae, reduced ectopterygoid and entopterygoid (or Mesopterygoid of Gosline, 1975) and the well developed metapterygoid are undoubtedly specialised features (figure 1A). The dorsal fin is inserted near the occiput so that the spines form a protective defense triangle. The palate dentition in most species is well defined than in other genera. The extent of lateral fenestra varies from species to species, and the temporal fossa is present in a reduced manner in all species (absent in *A. thalassinus*). The palatine articulation is from a single point of the ecto, meso and metapterygoid which is also a noteworthy feature (figure 1B).

Considering the most primitive of the 4 Indian genera, *Osteogeneiosus* qualifies for this position. The maxillary bone is produced in the form of a long semiosseous barbel (figure 2B) with a round base. This may be viewed by some as a very specialised character but this feature is not known in any of the Indian siluroid genera. The ethmoid bones are very much reduced. The lateral ethmoid is peculiar in shape and structure. The dorsal surface of this bone is honey combed and the bone is not as solid as is normally found in other Ariid genera (figure 2A). The frontal bones are very much reduced and in the form of thin, rod like structure anteriorly. The metapterygoid is smaller (figure 2C) than in *Arius*. Perhaps to facilitate movement of the semiosseous maxillary barbels, the extensor tentaculi muscle are well developed only in this genus whereas in other genera it is much reduced and absent in *Batrachocephalus*. Moreover, in *Osteogeneiosus* the palatines are well developed, stout and edentate. The extensor tentaculi, fills up the lateral fenestra and are also attached to the posterior half of the palatines. Both the meso and ectopterygoids are toothed and the metapterygoid is much smaller. It would seem that these modifications of the pterygoid bones are as a

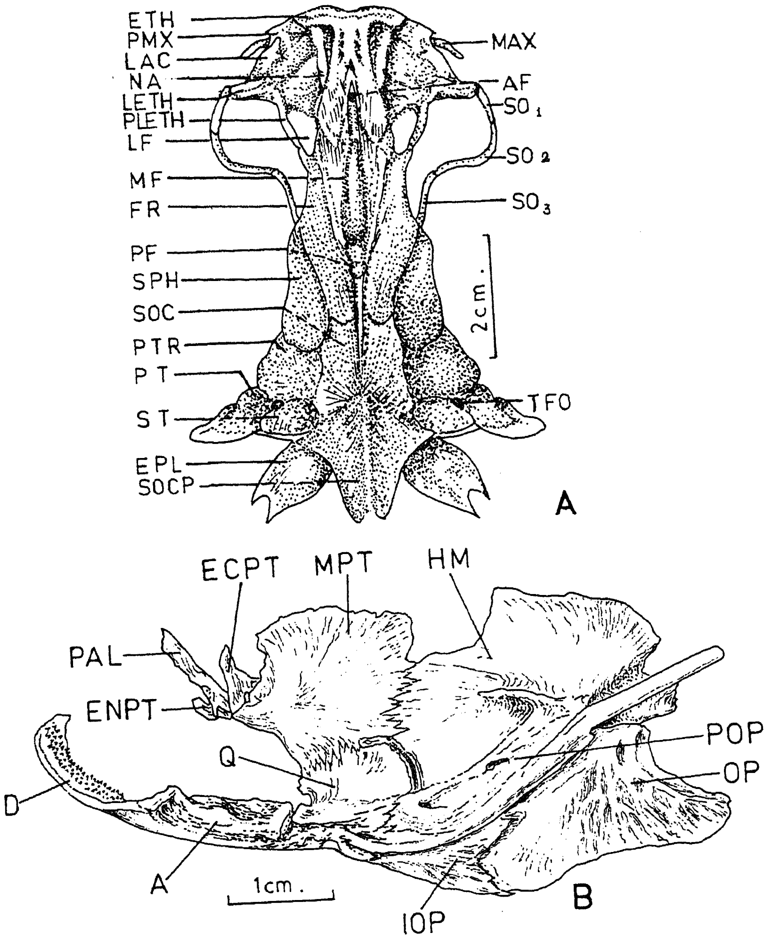


Figure 1. *Arius caelatus*. **A.** Dorsal view of skull. **B.** Hyomandibular suspensorium of *Arius caelatus*.

result of the change in the palato-maxillary mechanism discussed above. The dorsal and pectoral spines are well developed and the dorsal fin is inserted near the occiput as in *Arius*. Summing up it is evident that *Osteogeneiosus* is certainly the most primitive of the 4 genera. *Batrachocephalus* and *Ketengus* form one group and share some characters in common as the large sized eyes (figure 3) wide arcuate mouth, reduced maxillaries, reduced palatines etc. However of the two genera, *Ketengus* appears to be more primitive than *Batrachocephalus*. The lateral ethmoid is much reduced and is in the form of a paddle (figure 4A) without handle. Further, in *Ketengus* alone, the premaxillaries are well developed as a large plate (figure 4A) with a single row of teeth. This modification is again a unique feature found only in this genus of the Indian Ariidae. In *Batrachocephalus* the anterior end of the lateral ethmoid is squarish in shape, with the posterior arm interdigitating with the frontal (figure 5A). The cranial cavity extends to the ethmoid region and thus the cranium is *platybasic* in *Ketengus* a feature not present in the other 3 genera. The mesopterygoid bone is absent and its place seems to have been occupied by the enlarged metapterygoid (figure 5B).

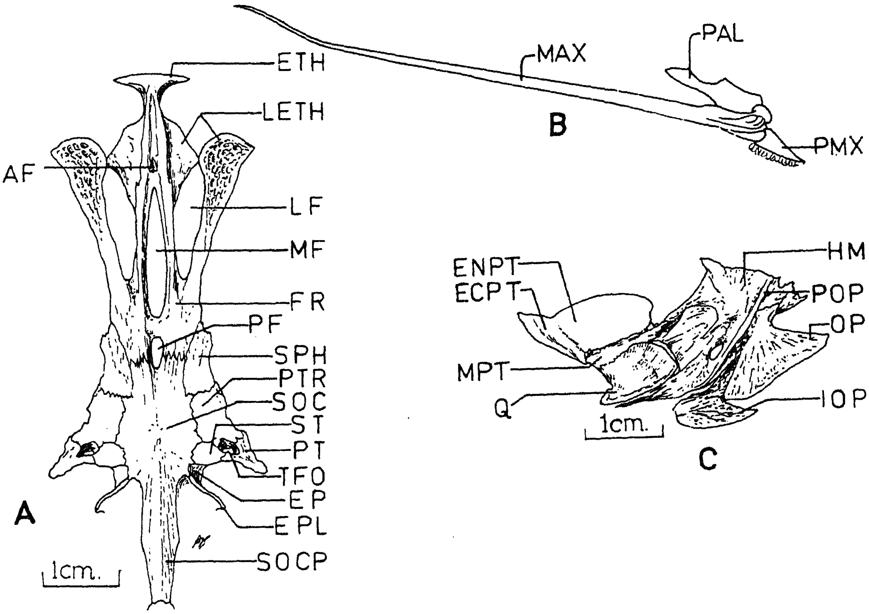


Figure 2. *Osteogeneiosus militaris*. **A.** Dorsal view of skull. **B.** Extension of maxillary bone. **C.** Hyomandibular suspensorium.

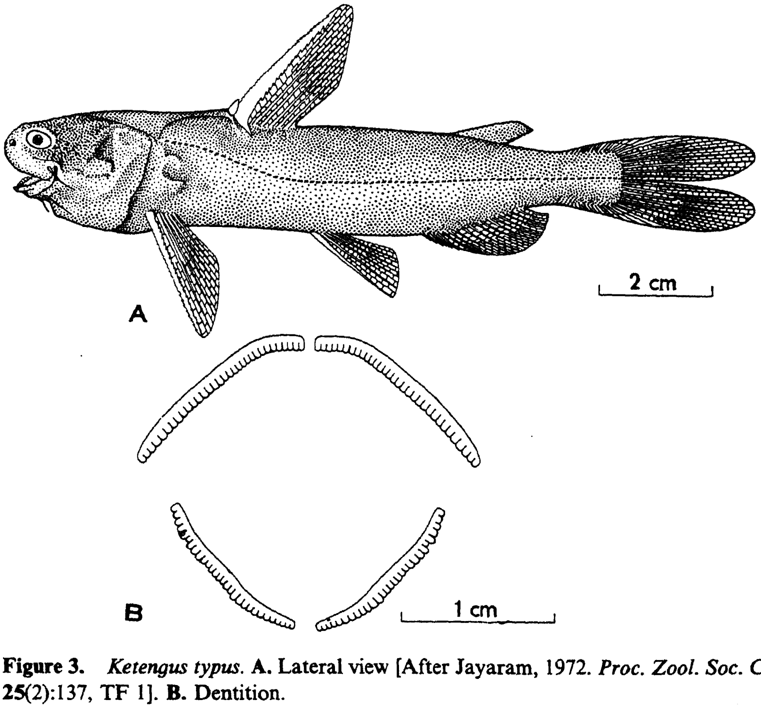


Figure 3. *Ketengus typus*. **A.** Lateral view [After Jayaram, 1972. *Proc. Zool. Soc. Calcutta,* **25**(2):137, TF 1]. **B.** Dentition.

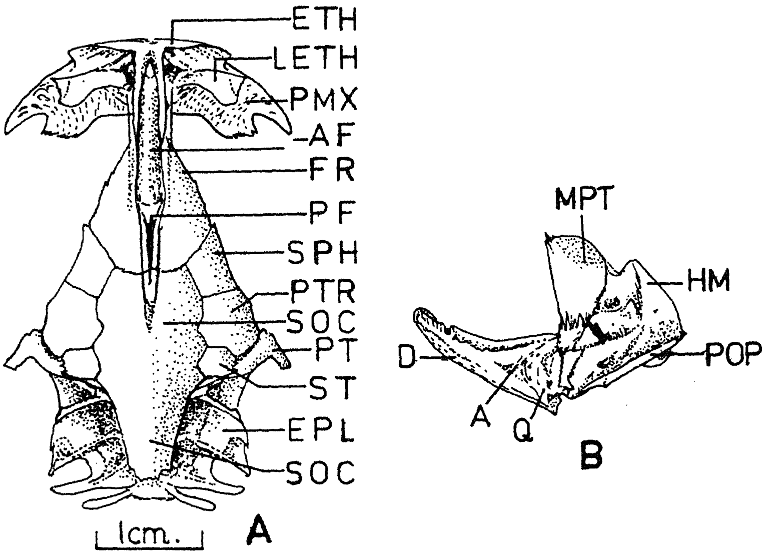


Figure 4. *Ketengus typus.* **A.** Dorsal view of skull. **B.** Hyomandibular suspensorium.

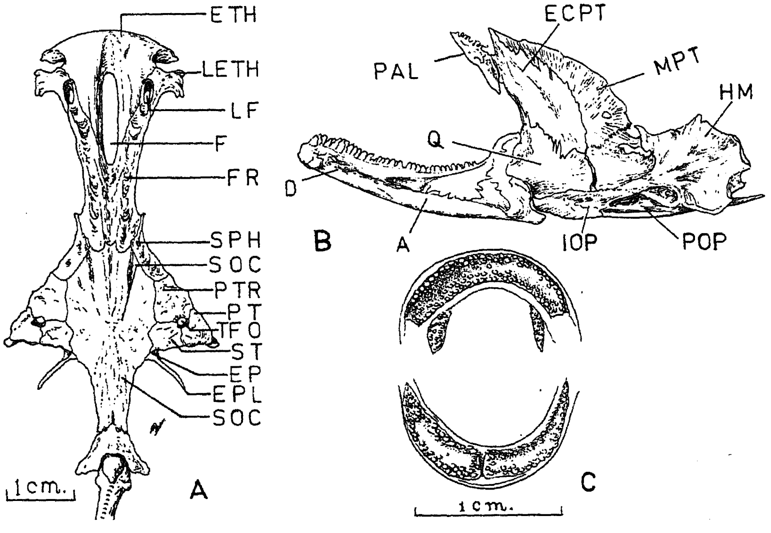


Figure 5. *Batrachocephalus mino* **A.** Dorsal view of skull. **B.** Hyomandibular suspensorium. **C.** Dentition.

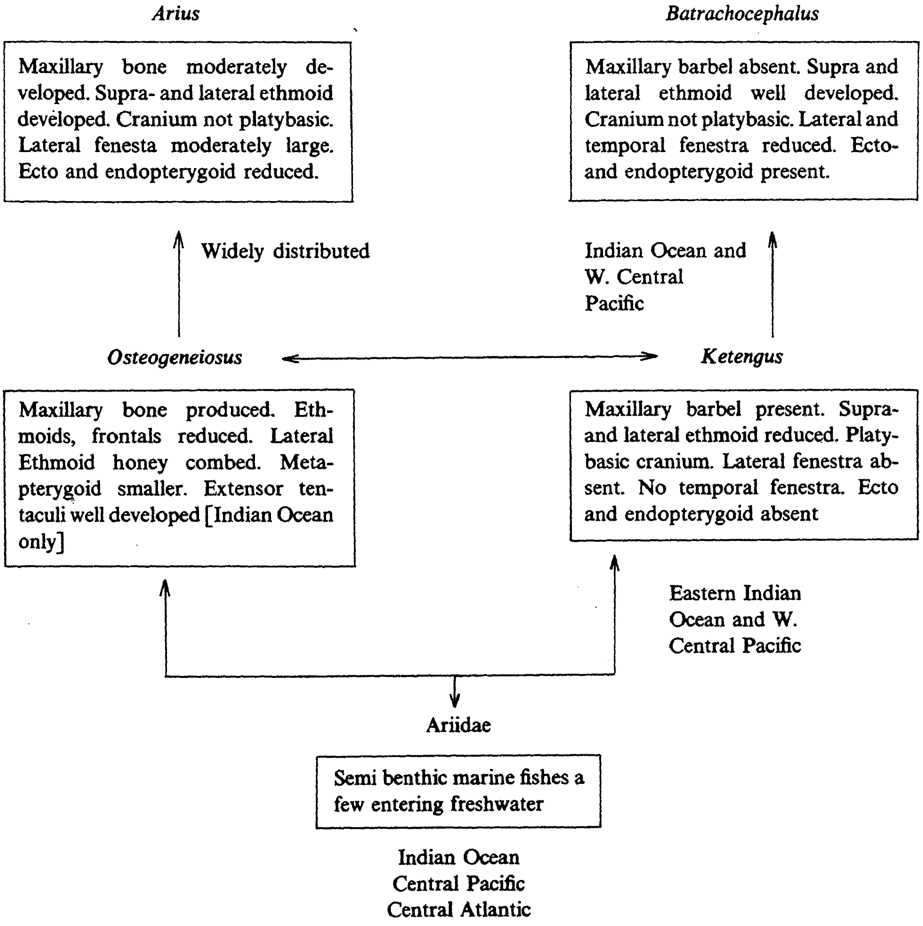
From the evidences adduced above it would appear that the Indian Ariidae have had two phyletic lines of evolution. Arius seems to have evolved from *Osteogeneiosus* like ancestors, whereas *Batrachocephalus* appears to be from *Ketengus* like fishes. No doubt *Arius* is much more advanced than all the other genera. It seems likely that an intermediate connecting link between *Ketengus* and *Osteogeneiosus* is or was existing about which information is lacking at this stage.

Table 2. A comparison of certain osteological differences in 4-genera of Ariidae from Indian waters.

Characters	*Arius*	*Osteogeneiosus*	*Batrachocephalus*	*Ketengus*
Maxillary	Moderately developed. Barbel present	Produced in the form of a long, semiosseous barbel with a round base	Much reduced. No barbel	Very much reduced. Barbel present
Ethmoid	Moderately developed, stout, interdigitating with the frontals posteriorly and the lateral ethmoids on either side	Much reduced, thin, narrow and short, interdigitating with the frontals posteriorly and the lateral ethmoids on either side	Well developed very wide interdigitating with the frontals posteriorly and the lateral ethmoid on either side	Much reduced, narrow, the posterior elongation being very thin interdigitating with the frontals and with the lateral ethmoid on either side
Lateral ethmoid	Well developed with a broad anterior end interdigitating with the ethmoid anteriorly and with the orbitosphenoid postero-ventrally	Peculiar in shape and structure. The dorsal surface is honey-combed externally. The anterior end is triangular, interdigitating with the ethmoid anteriorly and with the orbitosphenoid posteroventrally	Anterior end squarish in shape, interdigitating with the ethmoid anteriorly and with the orbitosphenoid posteroventrally	Much reduced, without any posterior elongation and is in the form of a paddle without an arm. Interdigitation is only with the ethmoid anteriorly
Cranial cavity	Not platybasic in the sense that it does not extend to the ethmoid region	Not platybasic	Not platybasic	Platybasic in the sense that the cranial cavity extends to the ethmoid region
Fontaneles	Three: anterior and posterior much reduced	Three: anterior smallest, middle one largest, posterior extending to supraoccipital	Single: posterior end not extending beyond the frontal bones	Two: anterior one very large

Lateral fenestra	Moderately large formed by the lateral ethmoid and the frontals	Very large, formed by the lateral ethmoid and the frontals	Much reduced	Absent
Frontal bones	Well developed	Much reduced almost rod like	Moderately developed	Reduced
Temporal fossa	Very small, in between supratemporal and pterotics and the post-temporal bones	Same as in *Arius*	Same as in *Arius*	No temporal fenestra but a short shallow, groove is present in between post-temporal, supratemporal and pterotics
Epioccipital	Well developed and prolonged	Not much developed or elongated as in *Arius*	Not much developed or elongated	Not much developed
Palatines (= Autopalatine of Gosline)	Moderately developed and stout. Without teeth. Articulating with the ecto- endo- and metapterygoids from a single point	Well developed and stout without teeth and overlapping endo- and ecto-pterygoids posteriorly	Very thin, much reduced without teeth overlapping endo- and ecto-pterygoids posteriorly	Very much reduced, thin without teeth overlapping metapterygoid and premaxillary
Premaxillary	Normal with many rows of teeth	Normal with many rows of teeth	Normal with many rows of teeth	Very broad with a single row of teeth
Suspensorium:				
Ectopterygoid	Reduced and shifted anteriorly. No teeth	Moderate, with teeth fused with entopterygoid	Well developed, without teeth	Absent
Mesopterygoid	Much reduced and lies underneath the posterior end of the palatines. No teeth	Moderate with teeth	Well developed, without teeth	Absent
Metapterygoid	Large and has occupied the position of ecto- and entopterygoids	Small	Small	Large and perhaps has occupied the position of ecto- and entopterygoids

Footnote to table 2.



Arius: Maxillary bone moderately developed. Supra- and lateral ethmoid developed. Cranium not platybasic. Lateral fenesta moderately large. Ecto and endopterygoid reduced.

Batrachocephalus: Maxillary barbel absent. Supra and lateral ethmoid well developed. Cranium not platybasic. Lateral and temporal fenestra reduced. Ecto- and endopterygoid present.

Osteogeneiosus: Maxillary bone produced. Ethmoids, frontals reduced. Lateral Ethmoid honey combed. Metapterygoid smaller. Extensor tentaculi well developed [Indian Ocean only]

Ketengus: Maxillary barbel present. Supra- and lateral ethmoid reduced. Platybasic cranium. Lateral fenestra absent. No temporal fenestra. Ecto and endopterygoid absent

Ariidae: Semi benthic marine fishes a few entering freshwater

Probable phyletic line of Indian Ariid genera

5. Conclusion

The interrelationships of the Indian genera as discussed above indicate clearly the ancient nature of the family which has diversified in two different phyletic lines. It also establishes the fact that *Arius* is more successful of the 5 genera and has been able to proliferate and reach wider areas than its ancestors in view of its specialised characteristics and better adaptability.

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Abbreviations used in the figures: A, Anguloarticular; AF, anterior fontanele; D, dentary; EP, epioccipital; ECPT, ectopterygoid; ENTP, mesopterygoid; EPL, epioccipital lamellae; ETH, ethmoid; FR, frontal; HM, hyomandibular; IOP, inter-operculum; LAC, lachrymal; LF, lateral fenestra; LETH, lateral ethmoid; MAX, maxillary; MF, median fontanele; MPT, metapterygoid; NA, nasal; OP, operculum; PAL, palatine; PF, posterior fontanele; PLETH, posterior process of lateral ethmoid; PMX, premaxillary; POP, preoperculum; PT, supracleithrum; PTR, pterotic; Q, quadrate; $SO_{1,2,3}$ suborbitals 1,2,3; SOC, supraoccipital; SPH, sphenotic; ST, posttemporal; SOCP, supraoccipital process, TFO, temporal fossa.

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2. Materials and methods

This presentation is based on our laboratory and field observations as well as pertinent information collected from literature. During the last few years, we have undertaken a series of physiological experiments to study the possibility of mass culture of *R. tigrina* (Marian 1982; Marian *et al* 1983; Marian and Pandian 1985a; Pandian and Marian 1985 a, b, c, d, e). During these studies, we observed the hatchability and survival of tadpoles of several ranids. To compare these laboratory data, we extended observations on these ranids in the temporary puddles situated in the vicinity of our school. Relevant published information was also collected from scientific journals and institutional reports as well as from our enquiry with several universities and frog collectors of our area.

3. Results

3.1 *Expansion of frog's habitat*

Frogs inhabit swampy and low-laying areas, especially the irrigated fields (Abdulali 1985). Of 304 million ha land available in India, 7·26 % (= 23 million ha) area was irrigated during 1951 (The Times of India 1982). To control flooding and to expand the irrigated agricultural land area, as many as 1554 large dams were constructed by the Government of India during the period from 1951 and 1980 (Agarwal *et al* 1982). Consequently, the expanse of irrigated land increased to 51 million ha; any effort made by man to increase the irrigated land area results also in the expansion of frogs habitat, which in turn, may enhance frog production. Thus within a period of about 30 years, the area of frog habitat and production has been inadvertently more than doubled. While we have no population census to support the increase of frog production, the parallel increasing trends obtained for the frog harvest by the export and education sectors (figure 1) provide a circumstantial evidence for the doubling of frog production during the last 30 years.

As it may be seen from table 1, the expansion of irrigated land area in the coastal states, where intensive harvest is being made for frog export, is comparable with the national average. Inland states like Punjab and Uttar Pradesh, in which frog harvest is restricted to education purpose have also more than doubled the irrigated land area. Hence, both at national level as well as in the coastal states, where frog is captured for export, the scope for frog production has doubled.

3.2 *Frog utilizing sectors*

Besides a constant fraction of frog population suffering predation in the natural habitats, recognizably large fractions of frog population were harvested and utilized in India from 1956 and 1967 by education and export sectors, respectively. Enquiries from several senior professors and students indicate that the syllabus of almost all Indian universities demands dissection of atleast 10 frogs by a biology student prior to his/her graduation. Hence a search was made to estimate the number of biology students graduated from Indian universities during the period from 1956–1980. The Times of

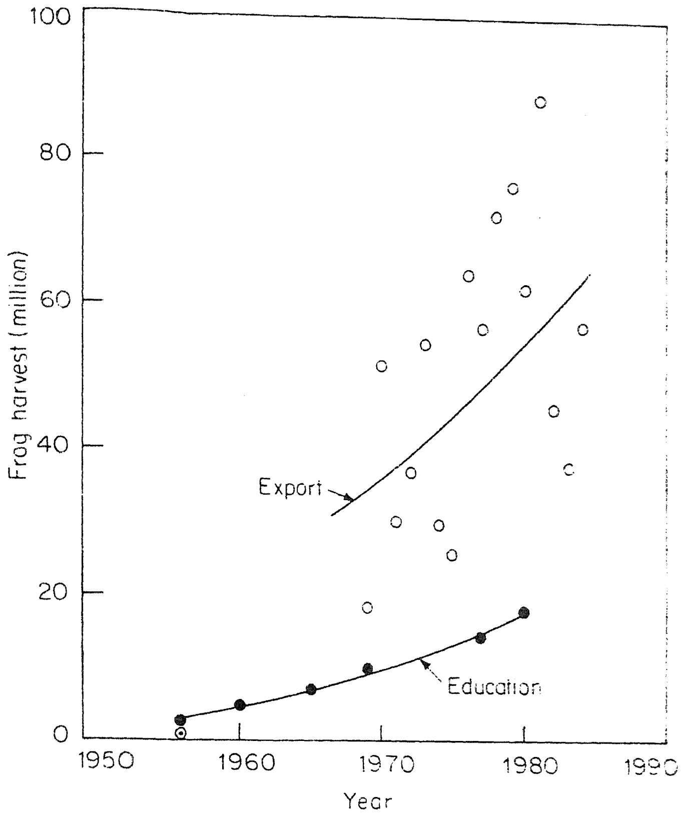

Figure 1. Increasing harvest of frogs by education and export sectors during the last 30 years. The symbol (⊙) represents the level of frog export initiated by Allen T Sherman (USA).

India (1982) reports that before independence, there were about 0·3 million students in 350 colleges affiliated to 20 universities in India. Today we have over 2 million students in 4500 colleges affiliated to 120 universities. Barring a singular exception of Aurangabad University, frog is continued to serve as the standard laboratory specimen in all these educational institutions for following reasons: (i) normally frogs do not feed under captivity and hence cost little to maintain them in the laboratory and (ii) the number of guide books available for the conduct of practicals on frog is far more than that available for any other laboratory animal. Understandably, frogs continue to serve as the most important laboratory animals for teaching biology students.

An assessment of the number and weight of frogs utilized in the biological education sector during the last 25 years was made by multiplying the number of biology students in India with 10 frogs each; this estimate indicates that the number of frogs harvested by the education sector increased from 2·6 million in 1956 to 18 million in 1980. A comparison of the trends obtained for the frog harvest by education and export sectors reveals that the first sector utilizes atleast 1/3 of that exported by the MPEDA sector (figure 1). It is therefore necessary to recognize that if frog populations have been overexploited in India, the export sector alone is not solely blamable, but the education

Table 1. Increase in irrigated land area in India as well as in its coastal and inland states during the period from 1956–1980 (from Times of India 1982)

Name of the State	Irrigated land area (in thousand hectares) 1951	1980	Increase (times)
India	23[b]	51[b]	2·2
Coastal states			
West Bengal	< 726	1541	> 2·0
Andhra Pradesh	2300	4691	2·2
Tamilnadu	2057	3819	1·9
Kerala	279	354	1·3
Karnataka	950	1718	1·8
Maharashtra	1431	2306	1·6
Gujarat[a]	740	1936	2·6
Inland states			
Punjab	2836	5506	1·9
Uttar Pradesh	< 4515	10575	> 2·3
Bihar	2279	3707	1·6
Madhya Pradesh	423	2413	5·7

[a] Frogs are also collected from Gujarat (see Abdulali 1985).
[b] Values given in millions.

sector has also been contributing its share to the alleged over-exploitation of frogs in India.

Unlike for the education sector, we have definite records for the number of frogs exported through MPEDA. Though frog export was commenced by Allen T Sherman, President of the International Fisheries Corporation, USA as early as in 1956, organized export of frog-legs was commenced in 1969, perhaps mostly through the efforts made by MPEDA (figure 1). The wide annual oscillations in the number of frogs exported certainly reflect the usual trend observed in capture fisheries (Jhingran 1975). Briefly the export sector increased the frog harvest from about 30 million in 1969 to 60 million in 1980. While the number of frogs harvested by export sector has consistently remained 3 times more than that harvested by education sector during 1969–1980, it is recognizable that both the export and education sectors almost doubled their harvest, i.e. export sector: from about 30–60 million; education sector from 10–18 million. The frog harvest by education sector has remained inconspicuous all these days; besides from the point of harvesting rate, i.e. doubling of frog's harvest in a decade, both these sectors have played an equal role.

Figure 2 shows the trend obtained for expanding frog's habitat area and the increasing cumulative frog harvest made by the education and export sectors of India. It may be noted that the rate, at which the cumulative frog harvest in India during 1970–1980 is far higher than the rate at which the frog's habitat, i.e. the irrigated area is increased. A projection of these trends certainly indicates that by 1981 our frog harvesting rate has exceeded the limit set by the habitat area and its frog production capacity. This is perhaps the reason for the decline in frog export since 1981.

Since we know the cumulative number of frogs harvested per annum and the annual expansion rate of irrigated area, it is possible to determine the rate of frog harvested in

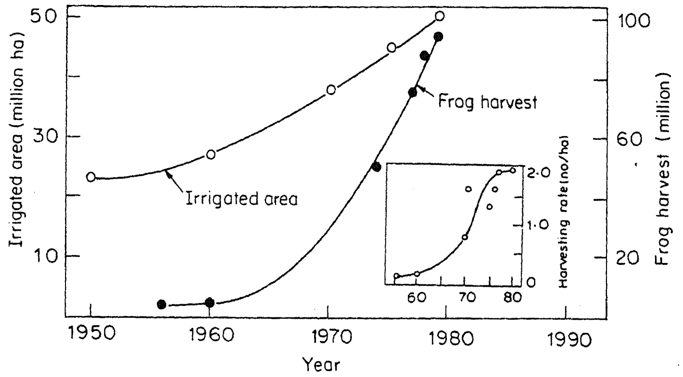

Figure 2. Expansion of irrigated area and increase in the cumulative frog harvest as a function of time. Window panel in the figure illustrates the harvesting rate of frogs as a function of time.

India. The window panel in figure 2 shows the trend obtained for the rate of frog harvest as a function of time. This analysis points out that the number of frogs harvested logarithmically increased from 0·2/ha in 1960 to 2/ha in 1978; subsequently the harvesting rate stabilized at that level showing that the maximum possible annual harvest of frog is only about 2/ha.

Mondal (1975) reported that the average size of frog exported by India is ≈ 100 g. From a series of experiments, Marian (1982) estimated the energy cost of living by an adult *R. tigrina* is 3·4 kJ/100 g/day, which is approximately equivalent to 1/16 of that reported for homeothermic mammals (Prosser 1973). McNab (1963) estimated the home range of a number of herbivorous and carnivorous mammals; using additional data obtained for the body weight and energy cost of living of these mammals, he showed that home range of these two groups of mammals linearly increases following with increasing body weight and that the home range of herbivore is smaller than that of a carnivore. Using the trend reported by McNab (1963) for the home range-carnivorous mammal relationship and considering the energy cost of living of the carnivorous frog, the home range of a 100 g *R. tigrina* was estimated to be in the range of 0·0357 ha; in other words, a ha of irrigated land may support 28 frogs. Since 2 frogs/ha can consistently be harvested per annum (figure 2, inset), it may be stated that a harvest of 7 % of the population from a habitat during a period of one year will neither deplete the frog population nor upset the ecological balance i.e. a harvest of 7 % of the frog population represents the optimum rate of utilization.

3.3 *Vector and pest control*

The role played by frogs in controlling the vectors like mosquitoes and pests like *Hieroglyphus banian, Oxya velox* may be assessed by observing the feeding habits or by analysing the stomach contents of freshly killed frogs. Sugumaran (1979) made a detailed study on the stomach contents of *R. tigrina* collected from the agricultural fields in and around Sholavandan, which is irrigated by the Vaigai river. His study is based on analysis of freshly killed *R. tigrina* collected around 2300 ha over a period of one year. He observed that insects and crabs form the major food items of *R. tigrina*, and the former constitute as much as 80% of the frog's food (table 2). He also grouped th different insects into the taxonomic order. Surprisingly he never recorded a mosquit nor even a dipteran (table 3). Incidentally, Pandian and Vivekanandan (1985) estimate the cost-benefit ratio of feeding, and concluded that a prey weighing 0·1% of th predator's body weight alone offers more energy to the predator than the energy co of a foraging, subduing and handling the prey. As the average weight of a mosquito i (< 2 mg wet weight) smaller than 0·05% weight of even a freshly metamorphose frogleg (> 1·8 g; Pandian and Marian 1985b,c,d; Marian and Pandian 1985a), it ma not be a surprise that neither mosquito nor any Diptera contribute to the food of frog Hence the question of using frog as a vector controlling agent is almost ruled out. I recent years, Abdulali (1985) made an intensive study on feeding habits of *R. tigrin* collected from Maharashtra state. While, the pest controlling ability of frog wa conclusively established, he too did not suggest a role for the frog in the control c vectors.

On the other hand, several important agricultural pests belonging to Orthopter Isoptera, Lepidoptera, Hemiptera and Coleoptera contributed the major bulk of th food organisms of frogs (table 2). Evidently, frogs do serve as an important controllin agent of agricultural pests. Frogs also consume a good percentage of crabs, which a known to destroy the agriculture land by their burrowing activity.

3.4 *Pesticides*

An average farmer in India depends more on pesticide application than using frog t control the pests in his agriculture fields. Since 1967–68, India has been producing a using as many as 55 varieties of pesticides; of them (i) DDT, (ii) BHC and (iii) carba (sevin) have been used extensively (David 1981). Application of pesticides poses constant threat to non-target organisms, especially frogs. During the rainy seaso pesticides sprayed on crops find their way to accumulate in temporary puddles surface run-off and sediment transport from the treated soil. Since the frogs bre

Table 2. Monthly variations in the (%) composition of food of *Rana tigrina* (from Sugumaran 1979; modified)

Month	Insects	Crabs	Miscellaneous[a]
January	96·7	2·1	1·2
February	99·4	0·0	0·6
March	97·5	0·9	1·6
April	96·5	1·2	2·3
May	99·4	0·6	0·0
June	47·4	0·0	52·6
July	98·3	1·1	0·6
August	47·8	8·7	43·5
September	43·3	10·0	46·7
October	82·5	7·0	10·5
November	76·9	7·7	15·4
December	83·3	5·6	11·1

[a] Earth worm, spider, centiped, slug, frog and snake.

Table 3. Monthly variations in the taxonomic composition of insects consumed by *Rana tigrina* (from Sugumaran 1979; modified).

Month	Odonata	Orthoptera	Isoptera	Lepidoptera	Hymenoptera	Hemiptera	Coleoptera
January	0	21	32	4	0	7	36
February	3	1	76	2	0	3	15
March	0	0	15	49	0	0	36
April	0	0	25	0	0	0	75
May	0	5	36	29	0	0	30
June	0	0	12	72	0	0	16
July	0	0	15	10	6	0	69
August	0	0	0	0	32	0	68
September	0	0	0	25	0	0	75
October	0	0	10	27	0	24	39
November	0	0	0	28	0	14	58
December	0	12	38	18	0	0	32

during rainy season and release their eggs in the temporary puddles, the developing tadpoles face the ill-effects of the accumulated pesticides. Marian *et al* (1983) have reported the deleterious effects of carbaryl on survival, growth and size of metamorphosing *R. tigrina*. Unfortunately, field survey on the deleterious effects of pesticides on frog population is totally wanting. It must be indicated here that theoretical models of the deleterious effects caused by pesticide application on target as well as non-target organisms, show that non-target organisms like frogs are ultimately eliminated rather than the pests in a given ecosystem (see Rosenzweig 1977). While the enthusiastic harvesting by the education and export sectors can at best make the frogs as endangered species, the consistent application of pesticides in the irrigated fields may cause the total depletion of frogs from the fields. Hence strategies for conservation of frogs should also consider the possibilities of minimizing pesticide application to conserve the non-target organisms including frogs.

3.5 *Scope for employment*

Frogs are usually caught during night with the aid of bright lamp and scooping nets, and are sold by the catchers to frog-cutting centres; the following morning the hind legs of these animals are cut off after they have been duly anesthetised under hygenic conditions in processing factories. However, frog collectors do not hesitate to cut off the hind legs more or less in a crude manner to reduce the weight of the frog bag. Photographic and cinematographic evidences of this kind of activities have been made, and on the strength of them, the SPCA of England, Germany and other countries have been pressurizing the ban of frog export from India. Legal enforcement of an embargo on frog export is difficult; for the collectors do not hesitate to smuggle the frogs to neighbouring countries to export them from there. Frogs are mainly collected from Tamilnadu, Kerala, Andhra Pradesh, West Bengal and Maharashtra (Mondal 1975). Enquiries with several frog collectors from our area suggest that the collection lasts for a period of 6 months during the monsoon and post-monsoon periods, and the harvesting rate is in the range of about 50 frogs/collector/night. Therefore, the harvesting of 80 million frogs for the export sector would provide scope for employment of 0·16 million people in rural areas. A recommendation of the ban on frog export from India should therefore simultaneously consider the problem of finding an alternate job for these rural folks, perhaps in aquaculture and ranching farms of frogs.

3.6 *Need for aquaculture and ranching*

In general most precipitation in India occurs during the brief spell of the monsoon period. Consequently there is considerable overlap in the breeding season among the anuran species (figure 3) and heavy competition for resource utilization among the tadpoles owing to the overlap of microhabitat (table 4). In general, tadpoles are highly specialized suspension feeders adapted for utilizing the rapid increase in primary production as a food source (Seale 1985). The availability of these resources are usually coupled to the monsoon (Haniffa and Pandian 1978, 1980). Being adapted to feed on the temporary resources, tadpoles suffer loss of body weight or heavy mortality, when food source begins to shrink or is suddenly depleted owing to the drying up of the

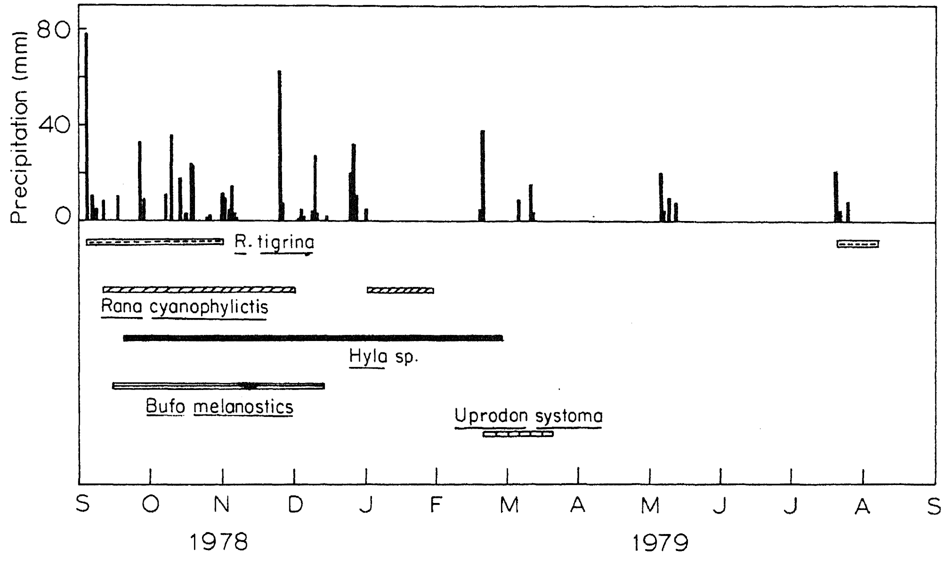

Figure 3. Upper panel: Daily changes in precipitation as a function of calendar months during 1978–1979. Lower panel: Spawning seasons of some anurans in the puddles of Madurai Kamaraj University as a function of calendar months. Horizontal bars represent the duration of spawning season by the respective species.

Table 4. Overlap of microhabitats of anuran larvae observed in the temporary puddles of Madurai Kamaraj University during 1977–1979.

Species pair comparison	Microhabitat overlap range
Rana tigrina – R. cyanophylictis	0·0168–0·3006
R. tigrina – Hyla sp.	0·0670–0·5646
R. cyanophylectis – Hyla sp.	0·2000–0·4706

Overlap in microhabitats is calculated using the formula:

$$\alpha jk = 2 \sum_i pij \cdot pik \left(\sum_i pij^2 + \sum_i pik^2 \right),$$

where $\alpha\, jk$ represents overlap of species *jk*, and *pij* the proportion of occurrence of species *j* in sweep *i*. Values 0 represent no overlap and 1 complete overlap.

puddles. Besides wide fluctuations in the commencement and distribution of monsoon reduce the successful emergence as well as the size of the emerging froglets (table 5).

The onset of monsoon triggers the spawning process in frogs; the clutch size of anurans ranges from 1280–1680 eggs/spawning and averages to 1474 ± 152 eggs (table 6). Our field observations show that the percentage of hatchability of *R. tigrina* is 60 ± 6%. Over 75–100% of tadpoles succumb to death during development due to infection or sudden drying-up of the puddles (Marian and Christopher 1985).

Table 5. Larval duration and froglet size of anurans emerging from the puddles of Madurai Kamaraj University during 1978–1979.

Species	Larval duration (days)	Froglet weight (mg)
R. tigrina	30–40	950–1100
R. cyanophylatis	70–75	1250–1500
Hyla sp.	25–30	150–200
Bufo melanostics	22–25	30–50
Uprodon systoma	20–26	300–350

Table 6. Clutch size and hatchability of anurans in the temporary puddles of Madurai Kamaraj University during 1977–1980.

	Egg (No.)			
Species	n	Range	x SD	Hatchability (%)
Rana tigrina	7	1280–1681	1474 ± 152	60 ± 6
R. cyanophlyctis	6	980–1538	1336 ± 212	67 ± 11
Hyla sp.	5	221–482	367 ± 86	79 ± 8
Uprodon systoma	6	858–955	910 ± 38	59 ± 4
Bufo melanostics	5	2000–5000	3500 ± 1224	92 ± 3

These field observations are neither adequate nor detailed but they point out the vicissitudes of environmental stress, to which the sensitive early stages of anurans are exposed in their natural habitats. On the other hand, reared under optimum laboratory conditions described by Pandian and Marian (1985a) more than 98% *R. tigrina* successfully complete metamorphosis and emerge. These laboratory and field observations clearly indicate the need for supporting aquaculture of frogs. The fact that frog harvest has begun to exceed the optimum harvestable limit since 1981, suggests the urgent need for massive inputs into frog culture and frog research in our country. There is vast scope for adoption of standard procedures like hypophysation, dry fertilization and mass rearing of tadpoles in high density cultures using tubifex as food. The most crucial problem in mass culture of tadpoles and adults is to find a 'live and kicking' prey in adequate quantity. Recently, Marian and Pandian (1984, 1985b) have described a simple procedure for the mass culture of tubifex using waste organic matter. Reared solely on *Tubifex tubifex*, a hatchling of *R. tigrina* attained a body weight of > 40 g in < 90 days requiring a feed of < 200 g *Tubifex* (Marian 1982). This is approximately the suitable size for release into the paddy fields to ensure conservation, and pests control as well as harvest by education and export sectors.

4. Discussion

Most conclusions drawn in this paper are not based on direct and adequate data. Therefore, the discussion is restricted to population census and aquaculture of frogs. Whereas the aquatic mode of life of Urodela (Merchant 1970; Fitzpatrick 1973; Burton and Likens 1975) and most anuran tadpoles (Licht 1974; Heyer 1976; Seale 1980) render the population census possible, the amphibious mode of life, the burrowing and aestivating habits of adult anurans make the population census a difficult task; added to these, the rapidity with which they dive into water or hop on land makes the task even more difficult. Besides no agency has come forward to identify population census of frog as priority area for funding. Therefore, it is not surprising to note the paucity of information on population census of frogs.

For want of information on mobility and home range of the Indian frogs, we have chosen to have an overall assessment of frog population census in a limited area. In the absence of pertinent information, we had to rely on the expanse of irrigated land area, i.e. the habitat of frog as an index of frog population and production. We have information on the number of frogs harvested by the export sector, but again we had to rely on an indirect assessment on the harvest made by education sector. We have brought to light that in recent years the education sector of India has become an important user of frogs and utilizes about 18 million frogs/annum. Culley (1973) reported that scholars and students in USA utilize about 15 million anurans annually (see also Gibbs *et al* 1971). Apparently biological education sector is an important user of anurans in many countries.

The need for aquaculture of frogs has been emphasized repeatedly (Culley 1973). However, frog culture has been found not a very successful enterprise (NAS 1974); the causes for this are: (i) incidence of heavy mortality of tadpole owing to cannibalism (Marian 1982; Marian *et al* 1985) (ii) the obligate requirement of proteinaceous feed for the grown-up tadpoles and (iii) the requirement of live, 'kicking' prey for the adult (Bardach *et al* 1972; Nace 1977). In India, Mondal (1975) attempted to mass culture frogs by providing illumination to attract phototropic insects. Unfortunately, no study was made on the quantity of attracted insects and the feed requirements of the frog population. Recently Marian and Pandian (1984, 1985b) have described a simple procedure to mass culture *Tubifex*, which is known to be the best feed ensuring maximum growth of *R. tigrina* (Marian 1982). As there is scope for solving the most critical problem, viz the frog feed, it should not be difficult to reventure into frog culture. Aquaculture of tadpoles may ultimately serve two purposes: (i) one for ranching programmes and (ii) harvesting for edible purposes. The former involves mass rearing tadpoles and release of juveniles into the irrigated field areas. Hence, it has the advantage of controlling agricultural pests but may render the scope for enthusiastic over-exploitation and killing of frogs under unhygenic conditions by unscrupulous collectors. On the other hand, mass culture of large frogs in aquacultural farms for education and export purposes may not contribute much to the control of agricultural pests but will still in the use of the carcass as feed and fertilizer. Both programmes require much scientific and governmental support, but the operation of these programmes in a judicious manner should provide maximum benefits.

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Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae)

M S M CHRISTOPHER* and S MATHAVAN
School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

*M/s Ram Bahadur Thakur (P) Ltd, Travancore Tea Estates Group, Siddharth Building, Cochin 682 003, India

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Abstract. Extensive field observations were undertaken to study the population dynamics of the larvae of *Catopsilia crocale* in the Madurai Kamaraj University campus during the years 1980–81 and 1981–82. Though oligophagous, *C. crocale* adopted 'monophagic strategy' for oviposition and larval feeding. Seasonal changes in the nitrogen content of the food *Cassia alata* leaf influenced the pupal/adult weight and oviposition. Commencement of rain triggered the reproductive activity and continued precipitation facilitated maximum egg deposition. Of total egg production in a year, about 80% was oviposited during northeast monsoon (September–December) and the rest during the south-west monsoon (May–August). Egg, I instar, V instar and pupa suffered more mortality than the other life stages. Of the total number of eggs laid, about 1·01 % emerged as adult and 98·99 % died during the process of larval and pupal development.

Keywords. Lepidopteran larvae; *Catopsilia crocale*; fecundity; life table; population dynamics.

1. Introduction

The occurrence of fluctuations in tropical butterfly populations has been reported by a number of workers (Ehrlich and Gilbert 1973; Watt *et al* 1979; Vats and Kaushal 1980). However, most of the workers have restricted their studies to the adult populations. Only a few publications report on fluctuations in the larval populations and the causative factors of the fluctuations (e.g. Hayes 1981). Environmental factors such as rainfall, temperature and relative humidity have been shown to influence the population either directly (Chew 1975; Watt *et al* 1977; Shapiro 1978a, b; Blau 1980) or through the host plants (Wolda 1978b; Mooney *et al* 1980, 1981; Lakhani and Dempster 1981).

While reviewing butterfly ecology, Gilbert and Singer (1975) stated that a few life tables have been compiled for butterflies, that too for a single generation (e.g. *Pieris rapae*; Harcourt 1966; Dempster 1967; Thomas 1974; *Lycaena dispar*; Duffey 1968). Recently, life table has been constructed for *Papilio polyxenes* (Blau 1980). Hayes (1981) made a detailed long term study on the population ecology and life table of the pierid butterfly *Colias alexandra*.

In the present study it has been attempted to analyse the factors influencing oviposition, larval population dynamics and life table of the butterfly *Catopsilia crocale*.

2. Materials and methods

2.1 *The study site*

The study site is situated within the Madurai Kamaraj University campus, Madurai (78° 10′ E and 9° 58′ N). Altitude is about 132·5 m. Madurai climate is monsoonic with an average annual rainfall of 400–700 mm. During the study period the mean maximum and minimum temperature varied from 38°C (June) to 21°C (January). It is a semi arid region consisting of a variety of complex diversified tropical fauna and flora. The area chosen for the study was about 7,500 m^2. The topography of the study site is given in figure 1.

2.2 *The organism*

Catopsilia crocale Cramer belongs to the Order Lepidoptera, Family Pieridae, subfamily Coliadinae. It is a tropical species, very commonly found in India, Ceylon and Burma (Wynter-Blyth 1935). The females are distinguished from the males by the presence of a black mark in the apex and the costa. In males, the apex and the costa are narrowly black, whereas in females they are broadly black in colour. Adult *C. crocale* occurs concomitantly with other related species like *C. pomona*, *C. pyranthae* and *C. florella* (Beeson 1941).

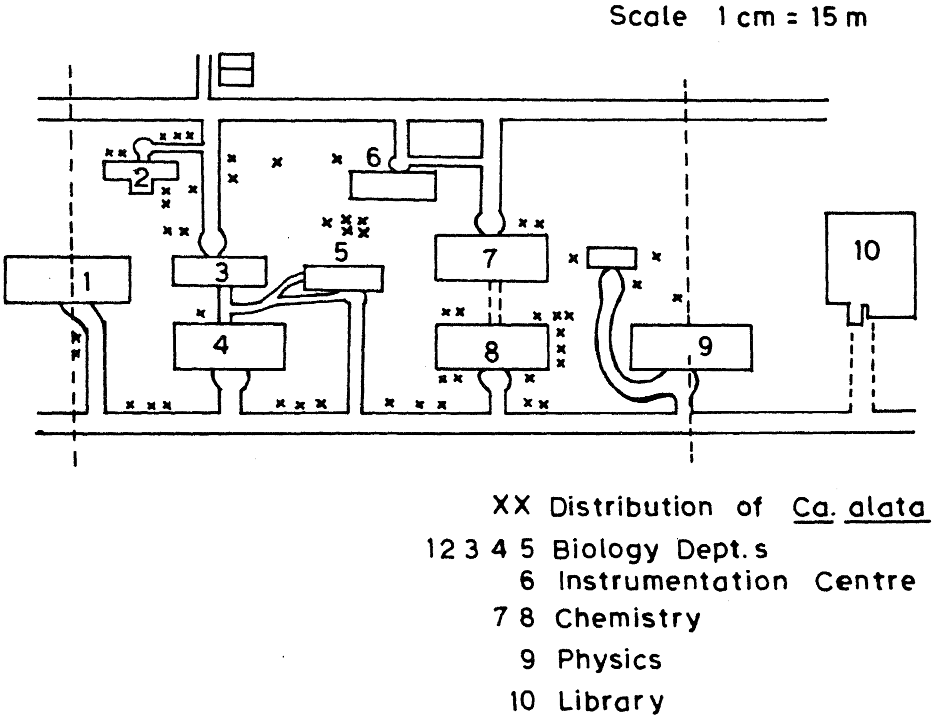

Figure 1. Topography of the study area.

C. crocale is a multivoltine species, breeds continuously throughout the year except in the dry season. However, adult population persists both in the dry and wet seasons of the year. *C. crocale* is oligophagous; it has been reported that the larvae utilize plants belonging to *Cassia* sp. such as *Ca. alata*, *Ca. siamea*, *Ca. nodosa*, *Ca. javanica* and *Ca. grandis* as food sources (Browne 1968). However, in the study area (habitat) though a variety of *Cassia* sp. were present, *C. crocale* females oviposited only on *Ca. alata* and larvae utilized the same plant as the potential food. Adults visited a number of plants belonging to various families for nectar.

2.3 *Food plant*

In India, *Ca. alata* is called as dadmurdum, which may be translated as herps-killer. *Ca. alata* is a soft woody shrub, belonging to the sub-family Cecalpiniae; cosmopolitan in distribution in the tropics and semi arid regions. These plants usually grow upto 3 m height. In the study area though plants of different height/age are distributed randomly, small plants (~ 1·5 m) outnumbered the large plants. At the beginning of summer, i.e. during the months of February–March they shed the old leaves and new buds sprout in May following the first precipitation. Maximum leaf production is synchronized with maximum precipitation i.e. during the months of September–November. Flowering commences during November/December. Since most of the conserved energy is utilized for flowering and seed production in the tall plants, leaf production is reduced after December.

2.4 *Egg and larval counting*

The study was conducted for two years during the period from January 1980 to January 1982. Total number of eggs oviposited on all *Ca. alata* plants in the study area during the two seasons were determined by direct counting method. Field observations were made on alternate days and each *Ca. alata* leaf was examined carefully. Eggs once counted were marked by a magic marker at the tip of the egg without causing any damage and hence the error of repeated counting was avoided. Eggs were deposited exclusively on the tender leaves and hence it was easy to locate them for counting.

For accurate censusing of larval population, the following method was adopted. Life stagewise number of larvae in each plant was counted on the day of observation. Since I, II, III and IV instars required only 2 days each to complete the feeding period, those at the II instar on the day of observation would have moulted into III instar on the subsequent observation, hence a particular stage larva was not counted twice for the same age. However, since final instar required about 2·5–3·0 days, total number of final instar larvae in each plant was counted in each observation; the increase or decrease of this larval stage in the subsequent observation was estimated by counting the newly formed pupa or newly moulted larvae. After a few weeks of careful regular field observations it became easier for us to census accurately.

2.5 *Life table*

To obtain complete information of mortality at each life stage of *C. crocale*, a life table was constructed using data from regular field observations on the number of eggs, larvae, pupae and adults. Causes of mortality were also recorded from the observations. From this, age specific life table was constructed for each month of the seasons. Life table data were subjected to 'key-factor' analysis (Varley and Gradwell 1960). k or killing factor was a concept originally by Haldane (1949) and applied by Morris (1959) and Varley and Gradwell (1960), as a statistical method for identifying the age specific causes of population change. The k value for the mortality of each life stage is the difference between the common logarithm of the number entering that stage and the subsequent one. The total generation mortality (K) was calculated by adding all the k-values. In order to know the monthly changes in the surviving individuals in each stage, survivorship curve was drawn by plotting the data obtained for monthly survivor in each life stage (lx) against the age (x).

Data on daily temperature (maximum and minimum), humidity (maximum and minimum) and rainfall during the study period were collected from the mini meteorological station of the School of Biological Sciences (courtesy: Unit of Animal Behaviour, School of Biological Sciences). From these data a monthly mean was calculated.

3. Results

The following plants have been reported to serve as food for *C. crocale* larvae: *Cassia alata*, *Ca. fistula*, *Ca. simona* and *Ca. javanica* (Browne 1968).

However, from extensive regular field observations, it was found that *C. crocale* oviposited on leaves of *Ca. alata* with nearly 100 % accuracy. Other *cassia* species in the same area did not attract the animal. Although the *C. crocale* larvae are considered to be oligophagous, the adults strictly follow the monographic type of oviposition. A similar type of oligophagous butterflies preferring monophagic type of oviposition has been reported for *Papilia machaon* (Wiklund 1974) and *Euphydryas editha* (Holdren and Ehrlich 1982).

C. crocale lays eggs singly on the leaves of *Ca. alata.* After landing on the plant, the female *C. crocale* requires about 4–6 seconds for the deposition of an egg. After egg deposition, she usually moves to another plant or another leaf of the same plant to deposit the next egg.

A careful observation revealed that though the food plants were distributed randomly, egg deposition was heavy on small plants and it was poor on large/tall plants. The height of the plant varied and a maximum height of 3·0 m was observed. Of the food plants present in the study area, about 20 % were above 2·5 m height. Surprisingly not even a single egg was observed in these plants during the study period of 25 months.

For plants of different heights, the number of eggs laid per plant were counted for a period of 5 days and are presented in table 1. The number of eggs laid per plant decreased with increasing height of the plant. A significant negative correlation was obtained and the slope of the regression, calculated are presented in figure 2 ($r = -0{\cdot}95$; $Y = 341 + 121{\cdot}5\,X$). With increasing nitrogen content of the leaf, total number of eggs deposited per plant increased ($Y = 237{\cdot}5 - 98{\cdot}7\,X$; $r = -0{\cdot}933$). Even in the small plants, *C. crocale* preferred mostly tender leaves for egg deposition.

The pattern of egg distribution on the leaf was observed and presented in table 2. Of the total 259 eggs observed in a chosen period, 126, 30, 103 eggs were found in the upper, lower and margin of the leaves, respectively. The eggs were observed

Table 1. The distribution of *C. crocale* eggs in relation to the leaf nitrogen content and height of *Ca. alata* plant.

Leaf nitrogen content (%)	Height of plant (m)	Eggs oviposited (No.)
3·00	3·00	—
3·01	2·40	16
3·04	2·00	40
3·41	0·45	90
4·00	0·90	101
4·60	0·35	240

Table 2. Oviposition preference of *C. crocale* on the *Ca. alata* leaf surface and egg survival in relation to oviposition site.

Position	Eggs oviposited (No.)	Hatched (No.)	Mortality (%)	Preference (%)
Total observed	259			
Upper surface	126	93	26·2	48·65
Margin	103	79	23·3	39·75
Lower surface	30	18	40·0	11·60

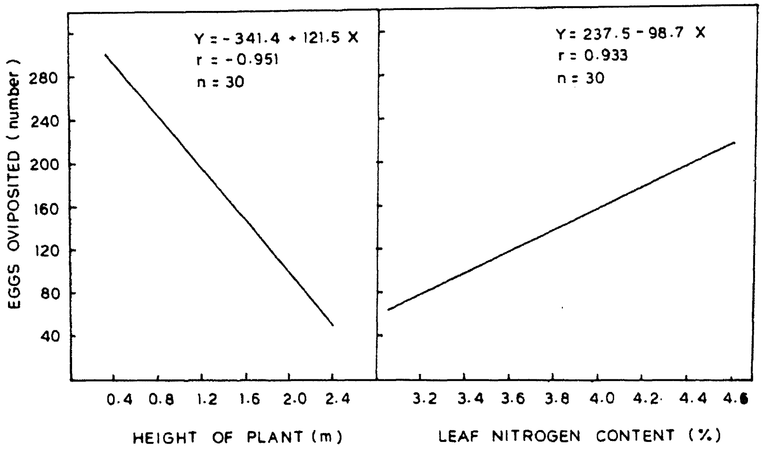

Figure 2. Oviposition of *C. crocale* as a function of plant height and nitrogen content of the leaf.

continuously and the hatchability was also recorded. It is interesting to note that eggs on the upper surface suffered a mortality of 26·2 % and those at the margin and lower surface suffered 23·3 and 40 % mortality, respectively. These differences, however, are not significant, but *C. crocale* preferred to deposit its eggs on the upper surface of the leaf.

Mean monthly data on rainfall, temperature and humidity recorded in the study area during the 25 month period is presented in table 3. From regular field observations number of eggs laid per week was calculated and related to rainfall for the corresponding period (figure 3). Oviposition was observed from January 1980 to January 1982. It is obvious from the figure that initiation of oviposition follows rains. *C. crocale* did not oviposit during the months of February, March, April 1980. First rain was recorded in April 1980. Following that *C. crocale* initiated oviposition in May. A precipitation of about 74 mm was recorded during the last week of May 1980 and consequently the oviposition increased in the following week. Subsequent absence of rain forced the *C. crocale* to reduce oviposition during June–August till the commencement of the northeast monsoon in September. The maximum oviposition of about 7869 eggs were recorded in the month of November 1980 and is clearly related to the maximum rainfall of 120 mm during the same period. Subsequently, as the precipitation decreased the oviposition also dwindled. Almost a similar pattern of egg laying was observed during 1981–82 season and the maximum was recorded in early November. From marking and recapture studies it was found that adults emerged during the end of the season i.e. during January 1980, survived upto April/May without much reproductive activity during the interim period.

Table 3. Monthly rainfall, temperature and relative humidity in the study area from January 1980 to January 1982.

Month	Rainfall (mm)	Temperature (°C)		Humidity (%)	
		Max	Min	Max	Min
January 1980	0·00	30·88	20·83	75·63	31·70
February	0·00	33·25	21·70	90·55	34·68
March	0·00	36·00	23·93	84·45	25·95
April	68·70	36·65	25·95	88·10	33·18
May	72·30	38·33	27·80	78·68	31·88
June	0·00	36·50	27·78	66·13	35·53
July	0·00	36·36	27·57	65·80	37·10
August	4·00	36·35	26·78	67·28	34·00
September	99·04	36·04	26·08	75·33	34·00
October	102·00	33·00	24·85	86·48	40·73
November	128·00	31·38	24·25	85·78	45·70
December	27·40	30·53	22·68	88·13	45·85
January 1981	1·10	31·53	21·30	84·40	33·78
February	2·50	33·55	21·33	83·85	28·25
March	20·30	35·55	24·45	90·80	32·45
April	37·50	37·70	26·98	86·63	32·70
May	43·90	37·60	27·13	79·50	32·65
June	0·00	35·10	27·03	75·78	40·90
July	110·80	34·87	25·95	78·19	41·38
August	26·70	35·21	25·44	77·19	37·35
September	424·00	32·95	23·63	92·00	47·13
October	271·90	31·29	23·13	96·55	53·84
November	250·20	27·80	21·00	95·41	60·00
December	10·40	28·20	20·60	91·85	47·54
January 1982	0·00	31·10	21·00	83·50	33·00

Figure 4 represents the data obtained on temperature (maximum and minimum) and number of eggs deposited per week during the study period. It is obvious from the figure that maximum oviposition coincides with minimum temperature. Regressions were developed relating fecundity to temperature and rainfall. It appears rainfall in the potent factor that regulate oviposition (figure 5).

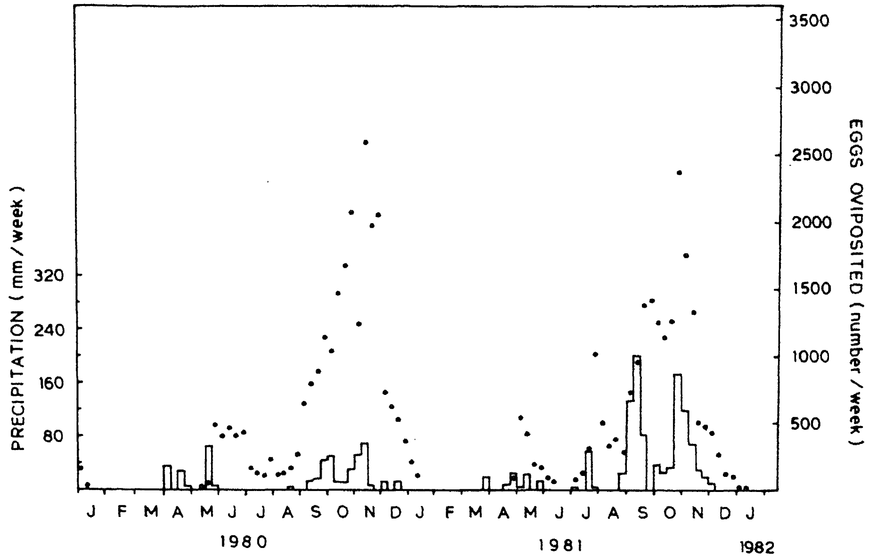

Figure 3. Mean weekly rainfall (histogram) and oviposition (●) per week in the study area for the seasons 1980–81 and 1981–82.

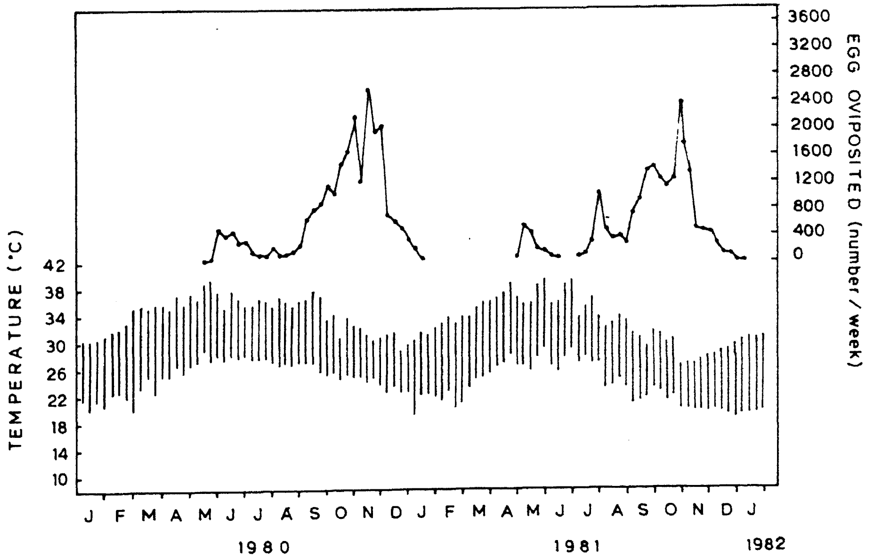

Figure 4. Relationship between weekly mean temperature (minimum and maximum) and oviposition of *C. crocale* in the study area for the seasons 1980–81 and 1981–82.

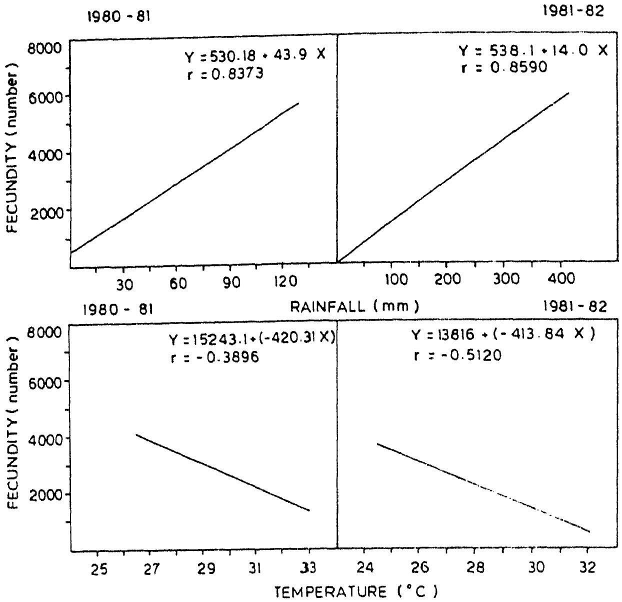

Figure 5. Regressions showing the relationship between rainfall fecundity and temperature and fecundity.

Table 4. Correlation between leaf nitrogen content and egg production during the year 1980.

Season	Nitrogen content (%)	Pupal weight (mg)	Egg production (No/female)
May	3·00	326 ± 14	132 ± 16
June	3·34	340 ± 15	163 ± 12
July	3·71	336 ± 12	166 ± 14
August	4·16	412 ± 18	230 ± 26
September	4·40	420 ± 10	259 ± 18
October	4·77	418 ± 13	260 ± 13
November	4·24	403 ± 21	256 ± 20
December	3·84	391 ± 15	200 ± 12

Monthly samples of *Ca. alata* leaves were collected (3rd and 4th leaf from the main bud; medium coarse) and analysed for nitrogen, lipid, carbohydrate and energy contents. Nitrogen, fat and energy content were maximum during the months of September–November and the minimum was recorded in the months of March/April. In other words, the materials in the leaves supporting growth of *C. crocale* were maximum during the peak oviposition period (monsoon period) and the minimum in the non-oviposition period. Quite interestingly, random samples of pupae collected from the field revealed a constant increase in mean pupal weight from the month of May–October and a decline thereafter (table 4). When leaf nitrogen content was regressed against the weight of the pupae collected from the field as well as the number of eggs obtained from the adults of these pupae, it became obvious that the leaf nitrogen content is highly correlated with the pupal and the adult weights and consequently with egg production (figures 6 and 7).

Using the basic data obtained every month on number of eggs and larvae surviving in each life stage of *C. crocale*, a life table was constructed for the seasons 1980–81 and 1981–82. First oviposition was observed in the month of May during 1980–81 season and in April during 1981–82 season.

Life tables constructed for each month are presented in tables 5 and 6. Based on the life tables, monthly survivorship curves of *C. crocale* were obtained for the seasons 1980–81 and 1981–82 (figures 8 and 9). It may be seen from the figures that survival was constantly low in egg and the I instar larvae throughout the season. Survival of the pupa and final instar was better during the early season than their survival in the middle or late seasons.

The survivorship curve obtained for *C. crocale* is a typical concave type; more than 70% of the animals died before they reached the mid larval stage (Price 1975). From the survivorship curve represented for the month of May 1980 and 1981, it is observed that over 96% of the individuals died before reaching the mid larval stage. With advancing season the mortality percentage decreased and during the month of December 1980, 65% of the individuals died before attaining middle larval stage.

Infertility of egg, feeding of the eggs by ants and the I instar larvae of *C. crocale* are the major factors of egg mortality. In the case of I instar in addition to the natural mortality, ant predators and rainfall are other factors that account for the loss of the larvae. During the II, III and IV larval stages, the major causes of mortality were predation by wasps, spiders, mantids, birds and ants. During the final larval and pupal stages the major loss was due to the heavy infection of bacterial and parasitic hymenoptera. Birds also prefer to predate upon the fully grown larva. Mortality of

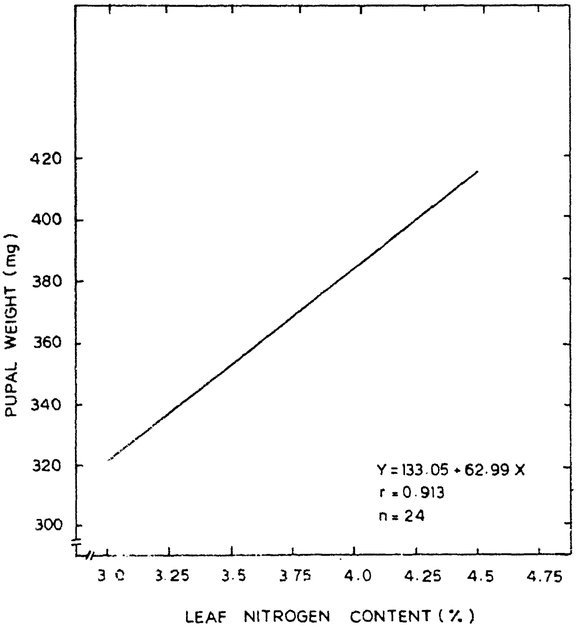

Figure 6. Pupal weight as a function of leaf nitrogen content.

Table 5. Age specific life table and key factor analysis for *C. crocale* population during different months of the season 1980–81.

Stage (x)	Number entering each stage (lx)	Mortality (dx)	dx as % of lx	dx as % of initial lx (cumulative)	log lx	k value K
May 1980						
Egg	554	392	70·76	70·76	2·7435	0·5340 k_1
I	162	111	68·52	90·79	2·2095	0·5019 k_2
II	51	30	58·82	96·21	1·7076	0·3854 k_3
III	21	4	19·05	96·93	1·3222	0·0918 k_4
IV	17	3	41·18	97·47	1·2304	0·0843 k_5
V	14	4	30·00	98·19	1·1461	0·1461 k_6
Pupa	10	1	14·28	98·38	1·000	0·0458 k_7
Adult	9				0·9542	
						1·7893 K
June 1980						
Egg	1506	1123	74·57	74·57	3·1778	0·5946 k_1
I	386	197	51·44	87·65	2·5832	0·3137 k_2
II	186	73	39·25	92·50	2·2695	0·2164 k_3
III	113	11	9·73	93·23	2·0531	0·0445 k_4
IV	102	14	13·73	94·16	2·0086	0·0641 k_5
V	88	28	31·82	96·02	1·9445	0·1663 k_6
Pupa	60	36	60·00	98·40	1·7782	0·3980 k_7
Adult	24				1·3802	
						1·7976 K
July 1980						
Egg	616	354	57·47	57·47	2·7896	0·3713 k_1
I	262	112	42·75	75·65	2·4183	0·2422 k_2
II	150	41	27·33	82·31	2·1761	0·1387 k_3
III	109	39	35·78	88·64	2·0374	0·1923 k_4
IV	70	27	38·57	93·02	1·8451	0·2116 k_5
V	43	13	30·23	95·13	1·6335	0·1564 k_6
Pupa	30	21	70·00	98·53	1·4771	0·5229 k_7
Adult	9				0·9542	
						1·8354 K
August 1980						
Egg	665	364	54·74	54·74	2·8228	0·3442 k_1
I	301	158	52·49	78·50	2·4786	0·3233 k_2
II	143	39	27·27	84·36	2·1553	0·1383 k_3
III	104	15	14·42	86·62	2·0170	0·0670 k_4
IV	89	30	33·71	91·13	1·9494	0·1785 k_5
V	59	19	32·20	93·98	1·7709	0·1688 k_6
Pupa	40	31	77·5	98·65	1·6021	0·6479 k_7
Adult	9				0·9542	
						1·8680 K
September 1980						
Egg	3468	1168	34·20			
I	2282	555	24·32			
II	1727	400	23·16			
III	1327	355	26·75			
IV	972	350	36·01			
V	622	326	52·41			
Pupa	296	262	88·51			
Adult	34					
October 1980						
Egg	6341	2929	46·19			
I	3412	867	25·41			
II	2545	723	28·41			
III	1822	607	33·32			
IV	1215	334	27·49			
V	881	481	54·60			
Pupa	400	340	85·00			
Adult	60					
November 1980						
Egg	7869	3504	44·53	44·53	3·8959	0·2559 k_1
I	4365	948	21·72	56·58	3·6400	0·1064 k_2
II	3417	826	24·17	67·07	3·5336	0·1201 k_3
III	2591	846	32·65	77·82	3·4135	0·1707 k_4
IV	1745	666	38·17	86·29	3·2418	0·2088 k_5
V	1079	639	59·22	94·41	3·0330	0·3895 k_6
Pupa	440	371	84·32	99·12	2·6435	0·8047 k_7
Adult	69				1·8388	
						2·0561 K

Table 5. (*Contd.*)

dx as % of initial *lx* (cumulative)	log *lx*	*k* value *K*	Stage (*x*)	Number entering each stage (*lx*)	Mortality (*dx*)	*dx* as % of *lx*	*dx* as % of initial *lx* (cumulative)	log *lx*	*k* value *K*
			December 1980						
34·20	3·5401	0·1818 k_1	Egg	2230	594	26·64	26·64	3·3483	0·1345 k_1
50·20	3·3583	0·1210 k_2	I	1636	246	15·04	37·67	3·2138	0·0708 k_2
61·74	3·2373	0·1144 k_3	II	1390	209	15·04	47·04	3·1430	0·0708 k_3
71·97	3·1229	0·1352 k_4	III	1181	410	34·72	65·43	3·0722	0·1851 k_4
82·06	2·9877	0·1939 k_5	IV	771	324	42·02	79·96	2·8871	0·2368 k_5
91·46	2·7938	0·3225 k_6	V	447	344	76·96	95·38	2·6503	0·6375 k_6
99·01	2·4713	0·9398 k_7	Pupa	103	83	80·58	99·10	2·0128	0·7118 k_7
	1·5315		Adult	20				1·3010	
		2·0086 *K*							2·0473 *K*
			January 1981						
46·19	3·8022	0·2692 k_1	Egg	590	350	59·32	59·32	2·7709	0·3907 k_1
59·86	3·5330	0·1273 k_2	I	240	90	37·50	74·58	2·3802	0·2041 k_2
71·27	3·4057	0·1452 k_3	II	150	49	32·67	82·88	2·1761	0·1718 k_3
80·84	3·2605	0·1759 k_4	III	101	35	34·65	88·81	2·0043	0·1848 k_4
86·11	3·0846	0·1396 k_5	IV	66	36	54·55	94·92	1·8195	0·3424 k_5
93·69	2·9450	0·3429 k_6	V	30	19	63·33	98·13	1·4771	0·4357 k_6
99·05	2·6021	0·8239 k_7	Pupa	11	7	63·64	99·32	1·0414	0·4393 k_7
	1·7782		Adult	4				0·6021	
		2·0240 *K*							2·1688 *K*

different life stages of *C. crocale* is presented in figure 10. The following important observations are made from the figure:

(i) Oviposition was observed for 9 months in a season; egg mortality was over 50 % in 6 months and was never less than 30 % during other periods.

(ii) First instar larvae suffered the maximum mortality in the early as well as in the late seasons. The mortality of the II, III and IV instar larvae averaged 33, 34 and 35 % respectively; it is far less than the mortality of egg, first or final instar larva.

(iii) Except the initial period of the season (2 or 3 months), the final instar and pupal mortality remained above 50 % and it averaged about 54 and 77 %, respectively.

These results show the existence of age dependent mortality of the larvae in the field. By pooling the data on the number in each life stage observed in the field, life table for the whole season was constructed and presented in table 7. The maximum potential natality was calculated by multiplying the total number of oocytes in each female with number of females recorded in the same month. In both the seasons the realized natality (egg) was less than the maximum potential natality. The females may not have deposited all the eggs they have in the ovary and some of the oocytes are resorbed. During the 1980–81 season 23,839 eggs were deposited in the study area. From this only 234 adults emerged. The total mortality was as much as 99.02 %.

During the 1981–82 season the total mortality was 98·96 % and it is not significantly different from the previous season. On observing the mortality of the eggs and larval

Table 6. Age specific life table and key factor analysis for *C. crocale* population during different months of the season 1981–82.

Stage (x)	Number entering each stage (lx)	Mortality (dx)	dx as % of lx	dx as % of initial lx (cumulative)	log lx	k value K	Stage (x)	Number entering each stage (lx)	Mortality (dx)	dx as % of lx
May 1981							August 1981			
Egg	1156	651	56·31	56·31	3·0630	0·3597 k_1	Egg	1498	445	29·71
I	505	218	43·17	75·17	2·7033	0·2454 k_2	I	1053	392	37·23
II	287	150	52·26	88·15	2·4579	0·3212 k_3	II	661	119	18·00
III	137	54	39·42	92·82	2·1367	0·2176 k_4	III	542	246	45·39
IV	83	36	43·37	95·93	1·9191	0·2470 k_5	IV	296	117	39·53
V	47	7	14·89	96·54	1·6721	0·0700 k_6	V	179	65	36·31
Pupa	40	19	47·50	98·18	1·6021	0·2799 k_7	Pupa	114	98	85·96
Adult	21				1·3222		Adult	16		
						1·7408 K				
June 1981							September 1981			
Egg	156	78	50·00	50·00	2·1931	0·3010 k_1	Egg	4493	1435	31·94
I	78	46	58·97	79·49	1·8921	0·3869 k_2	I	3058	1233	40·32
II	32	16	50·00	89·74	1·5052	0·3011 k_3	II	1825	822	45·04
III	16	8	50·00	94·87	1·2041	0·3010 k_4	III	1003	451	44·97
IV	8	1	12·50	95·51	0·9031	0·0580 k_5	IV	552	190	34·42
V	7	2	28·57	96·79	0·8451	0·1461 k_6	V	362	226	62·43
Pupa	5	3	60·00	98·71	0·6990	0·3980 k_7	Pupa	136	88	64·71
Adult	2				0·3010		Adult	48		
						1·8921 K				
July 1981							October 1981			
Egg	1567	748	47·73	47·73	3·1951	0·2818 k_1	Egg	6027	1649	27·36
I	819	492	60·07	79·13	2·9133	0·3988 k_2	I	4378	1229	28·07
II	327	159	48·62	89·28	2·5145	0·2892 k_3	II	3149	804	25·53
III	168	86	51·19	94·77	2·2253	0·3115 k_4	III	2345	936	39·91
IV	82	20	24·39	96·04	1·9138	0·1214 k_5	IV	1409	341	24·20
V	62	12	19·35	96·81	1·7924	0·0934 k_6	V	1068	578	54·12
Pupa	50	33	66·00	98·92	1·6990	0·4686 k_7	Pupa	490	430	87·76
Adult	17				1·2304		Adult	60		
						1·9647 K				

dx as % of initial lx (cumulative)	log lx	k value K	Stage (x)	Number entering each stage (lx)	Mortality (dx)	dx as % of lx	dx as % of initial lx (cumulative)	log x	k value K
			November 1981						
29·71	3·1755	0·1531 k_1	Egg	6003	2800	46·64	46·64	3 7784	0·2728 k_1
55·87	3·0224	0·2022 k_2	I	3203	1693	52·86	74·85	3·5056	0·3266 k_2
63·82	2·8202	0·0862 k_3	II	1510	362	23·97	80·88	3·1790	0·1190 k_3
80·24	2·7340	0·2627 k_4	III	1148	242	21·08	84 91	3·0600	0·1029 k_4
88·05	2·4713	0·2184 k_5	IV	906	200	22·08	88·24	2·9571	0·1082 k_5
92·39	2·2529	0·1960 k_6	V	706	326	53·28	93·67	2·8489	0·2680 k_6
98·93	2·0569	0·8528 k_7	Pupa	380	322	84·74	99·03	2·5809	0·8175 k_7
	1·2041		Adult	58				1·7634	
		1·9714 K							2·0150 K

Table 6. (*Contd.*)

dx as % of initial *lx* (cumulative)	log *lx*	*k* value *K*	Stage (*x*)	Number entering each stage (*lx*)	Mortality (*dx*)	*dx* as % of *lx*	*dx* as % of initial *lx* (cumulative)	log *x*	*k* value *K*
			December 1981						
31·94	3·6525	0·1671 k_1	Egg	1903	504	26·48	26·48	3·2794	0·1336 k_1
59·38	3·4854	0·2241 k_2	I	1399	291	20·28	41·78	3·1458	0·1013 k_2
77·68	3·2613	0·2600 k_3	II	1108	368	33·21	61·11	3·0445	0·1753 k_3
87·71	3·0013	0·2594 k_4	III	740	310	41·89	77·40	2·8692	0·2357 k_4
91·94	2·7419	0·1832 k_5	IV	430	240	55·81	90·01	2·6335	0·3547 k_5
96·97	2·5587	0·4252 k_6	V	190	126	66·32	96·64	2·2788	0·4726 k_6
98·93	2·1335	0·4523 k_7	Pupa	64	47	73·44	99·10	1·8062	0·5758 k_7
	1·6812		Adult	17				1·2304	
		1·9713 *K*							2·0490 *K*
			January 1982						
27·36	3·7801	0·1388 k_1	Egg	504	281	55·75	55·75	2·7024	0·3541 k_1
47·75	3·6413	0·1431 k_2	I	223	82	36·77	72·02	2·3483	0·1991 k_2
61·09	3·4982	0·1281 k_3	II	141	45	31·91	80·95	2·1492	0·1689 k_3
76·62	3·3701	0·2212 k_4	III	96	36	37·50	88·09	1·9823	0·2041 k_4
82·28	3·1489	0·1203 k_5	IV	60	29	48·33	93·85	1·7782	0·2868 k_5
91·87	3·0286	0·3384 k_6	V	31	10	32·36	95·83	1·4914	0·1692 k_6
99·00	2·6902	0·9120 k_7	Pupa	21	17	80·95	99·21	1·3222	0·7201 k_7
	1·7782		Adult	4				0·6021	
		2·0019 *K*							2·1003 *K*

stages of 1980–81 season, it became apparent that about 40 % of the eggs failed to hatch. The mortality in the first 4 larval stages averaged to about 30 % while the V instar and pupae suffered 57 and 83 %, respectively. During 1981–82 season it ranged from 36 % in the egg to 30 % in the IV instar. However, V instar (50·15 %) and pupae (81 %) showed the maximum percentage of mortality. This clearly depicts the low survivorship of the final instar and pupae for the season 1980–81 and 1981–82. Analysis of *k* factor reveals that the maximum *k* value of above 0·7 was observed for the pupal stage in both the seasons and the second maximum was observed for the V instar (about 0·3).

4. Discussion

Even though females of *C. crocale* were confronted with an array of host plants such as *Ca. fistula*, *Ca. siamea*, *Ca. javanica* and *Ca. grandis*, they restricted oviposition to the leaves of *Ca. alata* which appears to be a case of 'monophagic strategy'. Females clearly preferred to oviposit on the leaves of *Ca. alata* and avoided plants belonging to other *Cassia* species, though these plants are present in a similar topography along with *Ca. alata*. This type of monophagic strategy is found in many oligophagous butterflies such as *P. machaon* (Wiklund 1974, 1975), *E. editha* (Singer 1971; Holdren and Ehrlich 1982) and *Heliconius melpomene* (Smiley 1978). Slobodkin and Sanders (1969) and Wiklund (1974) stated that monophagic strategy would be favoured by species living in

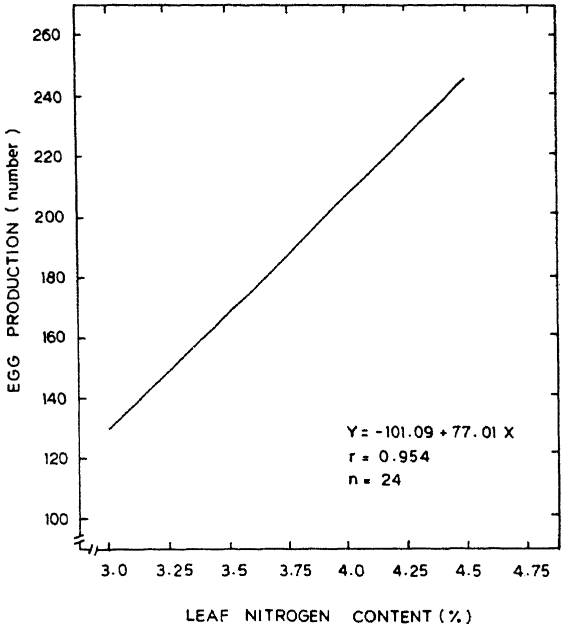

Figure 7. Egg production as a function of leaf nitrogen content.

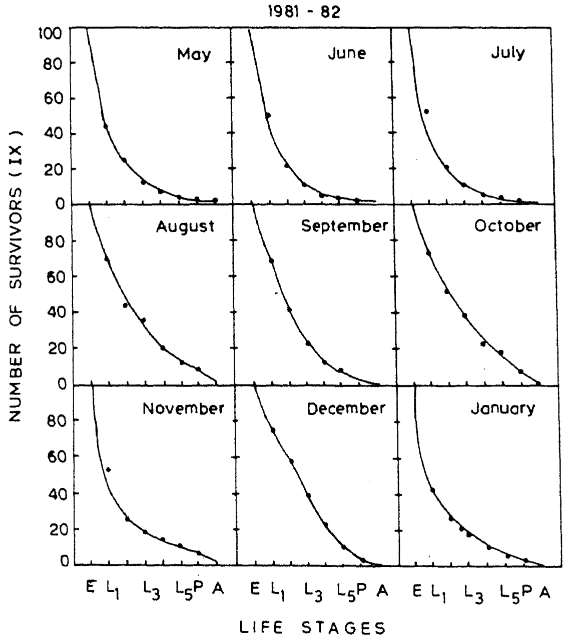

Figure 8. Survivorship curve of different life stages of *C. crocale* population during the season 1980–81 (E, egg; L_1, L_2, L_3, L_4, L_5, larval stages; P, pupa; A, adult).

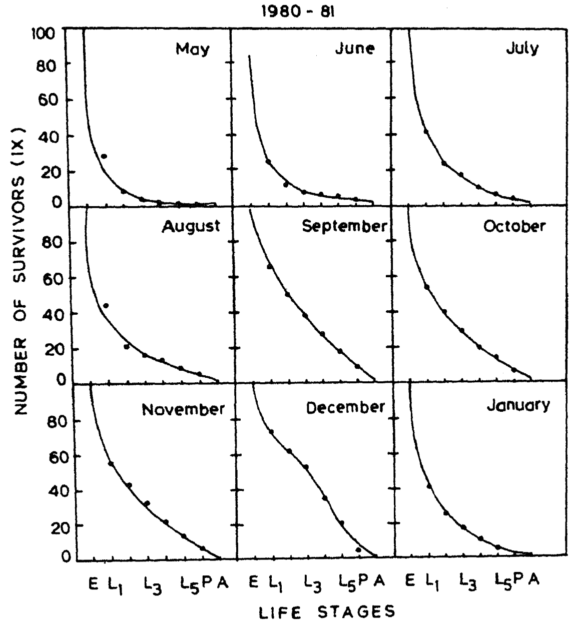

Figure 9. Survivorship curve of different life stages of *C. crocale* population during the season 1981–82 (E, egg; L_1, L_2, L_3, L_4, L_5, larval stages; P, pupa; A, adult).

predictable environments (habitats) which allow specialization to narrow niches. Moreover oligophagous species having adopted the monophagic strategy will predominantly utilize perennial plant species or species which are abundant in the habitat (Wiklund 1974).

Similarly, Smiley (1978) and Gilbert (1978) suggested that monophagic strategy observed in the butterfly *H. melpomene* might have originated, since food plants might differ in their suitability for oviposition, larval growth and survival or for reasons other than biochemical or nutritional differences. In addition, ecological factors such as predation might have affected the evolution of monophagy in these butterflies. Holdren and Ehrlich (1982) concluded that the monophagic strategy found in *E. editha* towards *Castilleja linarifolis* plant is not because of their biochemical make-up but because of their ecological characteristics, primarily their phenologies. Courtney (1983) has further showed that evolution of monophagous relationship is possible even when alternative host plants are present. The oviposition choice of *C. crocale* females seems to agree with the Hopkins host selection principle (Hopkins 1917); it states that a female's oviposition preference is biased in favour of the plant species which she fed upon as larva.

C. crocale females identified young *Ca. alata* plants for potential egg laying and carefully avoided the older (taller) plants. It may be due to the following reasons.

(i) The young plants have more number of tender leaves and thus supported the freshly emerged larvae better than the older plants.

(ii) In small plants, the young tender leaves are exposed to sunlight for a maximum period of time in a day and attract ovipositing females. In older plants, the best

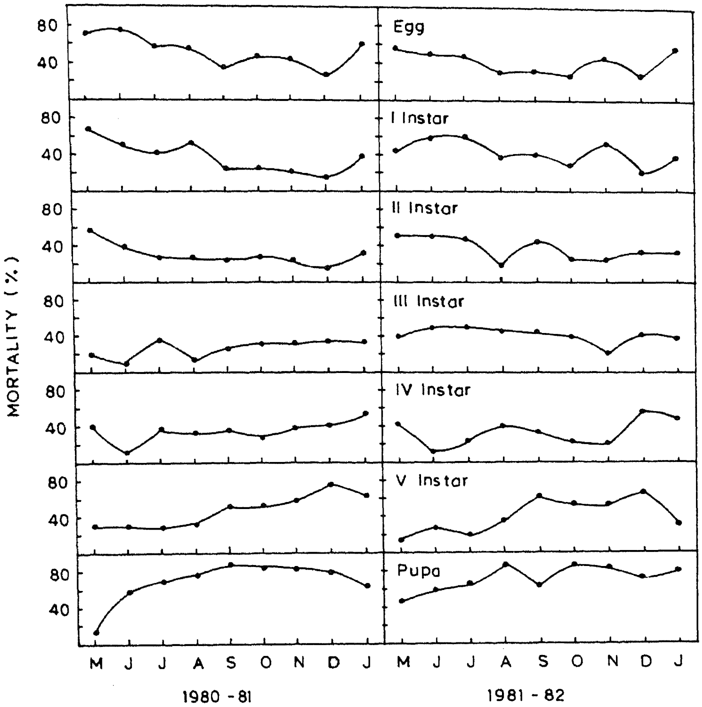

Figure 10. Percentage mortality of egg, larval and pupal stages of *C. crocale* during different months of the study period 1980–81 and 1981–82. Mortality caused by all the killing factors are combined.

leaves are shaded and the animals avoid shaded regions for egg laying (e.g. Chew 1974; Rausher 1979; Courtney 1981).

(iii) Leaves of the young plants contain more of digestible nitrogen and less of undigestible fibre; whereas the reverse is true in the leaves of older plants. Correlation between increasing plant age and decreasing leaf nitrogen has been reported in many plants (e.g. Mooney *et al* 1981).

C. crocale females did not choose all the *Ca. alata* plants of similar size for egg laying. Though a number of similar plants (physiologically same age) existed in the same area, the butterflies preferred certain plants for egg laying. The reason for this preference is not known. This is in contrast to the observations reported by Rothschild and Schoonhoven (1977). Wherein *P. brassicae* and *P. rapae* avoided egg bearing plants for further egg laying. A similar observation was also made by Wiklund and Ahrberg (1978) for *Anthocaris cardenines* (see also Williams and Gilbert 1981).

Seasonal fluctuation in the egg production of *C. crocale* clearly coincides with the fluctuation in the energy and chemical composition of food plant. Nitrogen content of the plant is vitally important element for herbivores (Mattson 1980; Slansky and Feeny 1977) in addition to leaf water content (White 1976; Scriber 1977). With the onset of southwest monsoon, new *Ca. alata* plants appeared and/or young *Cassia* plants produced more tender leaves in the same cohort. Freshly sprouted leaves contain maximum nitrogen content. It was maximum in the peak monsoon period and declined

Table 7. Age specific life table and key factor analysis for *C. crocale* during 1980–81 and 1981–82 seasons

X	*lx*	*dx*	*dx* as % of *lx*	*dx* as % of initial *lx* (cumulative)	log value	*k* value	*k*
1980–81							
PN	55534						
Egg	23839	10796	45·29	45·29	4·3773	0·2619	k_1
Instar I	13043	3284	25·18	59·06	4·1154	0·1260	k_2
Instar II	9759	2390	24·49	69·09	3·9894	0·1220	k_3
Instar III	7369	2322	31·51	78·83	3·8674	0·1644	k_4
Instar IV	5047	1784	35·35	86·31	3·7030	0·1894	k_5
Instar V	3263	1873	57·40	94·17	3·5136	0·3706	k_6
Pupa	1390	1156	83·16	99·02	3·1430	0·7738	k_7
Adult	234				2·3692		
						2·0081	*K*
1981–82							
PN	56680						
Egg	23396	8649	36·97	36·97	4·3691	0·2004	k_1
Instar I	14747	5693	38·60	61·30	4·1687	0·2119	k_2
Instar II	9054	2854	31·52	73·50	3·9568	0·1644	k_3
Instar III	6200	2371	38·24	83·63	3·7924	0·2093	k_4
Instar IV	3829	1177	30·74	88·66	3·5831	0·1595	k_5
Instar V	2652	1352	50·98	94·44	3·4236	0·3097	k_6
Pupa	1300	1057	81·31	98·96	3·1139	0·7283	k_7
Adult	243				2·3856		
						1·9835	*K*

PN, Potential natality.

as the rainy season declined. As the plants started flowering, maximum nitrogen was channelled for reproductive part of the plants. Mooney *et al* (1981) reported that leaf nitrogen of the Californian shrub *Deplacus aurantiacus* declined as the flowering commenced. A similar case is true for *Ca. alata*. It is assumed that leaf quality of *Ca. alata* determines the reproductive potential of *C. crocale*. Wiklund and Person (1983) showed that females of the speckled wood butterfly, *Pararge aegeria* lay eggs that decrease in size over the oviposition period. However, we did not make such observation during the oviposition of *C. crocale*.

In southern India, the southwest monsoon starts from April and continues to August and the northeast monsoon commences in September extending to December. During the 1980–81 season, the first rain (29 mm) was recorded on 5th April 1980. Following this precipitation, the first oviposition was observed on 4th May 1980. During the southwest monsoon total precipitation was 145 mm. Temperature ranged between a maximum of 37°C and a minimum of 27°C and the humidity between 73 and 34 % rh. Total number of eggs produced during the same period was 3341. It represents 14 % of the total egg production of the 1980–81 season. During the same year the northeast monsoon brought about 356 mm rain and 83 % of the total egg production was

Table 8. Oviposition of *C. crocale* population, rainfall, temperature (minimum and maximum) and humidity (minimum and maximum) during southwest and northeast monsoon in the study area during the season 1980–81 and 1981–82.

Monsoon period	Eggs oviposited (No.)	Rainfall (mm)	Temperature (°C) Max	(mean) Min	Humidity Max	(mean) Min
1980–81						
April–August (southwest monsoon)	3341	145·0	36·84	27·18	73·20	34·34
September–December (northeast monsoon)	19908	356·4	32·74	24·47	83·93	41·57
1981–82						
April–August (southwest monsoon)	4377	218·9	36·10	26·51	79·46	37·00
September–December (northeast monsoon)	18426	956·5	30·06	22·09	93·95	52·13

recorded during this period (table 8). Almost a similar pattern of rainfall and egg deposition was obtained during 1981–82 season. Precipitation was 219 and 956 mm during the southwest and northeast monsoons, respectively. Of the total oviposition during the season 18 % was in the southwest and 82 % in the northeast monsoon. Even within the monsoon period, observation on oviposition (per week) fluctuated in relation with the increased or decreased rainfall for the corresponding period.

Growth of *C. crocale* population was initiated with the commencement of southwest monsoon and reached the maximum density during the northeast monsoon. During the non-monsoon period no appreciable rainfall was recorded; further, no significant variation in temperature and humidity was observed. From these observations, it may be concluded that rainfall triggered and governed the initiation and continuation of oviposition in *C. crocale*, while the other factors like temperature and humidity facilitated the same indirectly.

Shapiro (1978b) clearly stated that no information is available on the environmental regulation of life history phenomena of tropical butterflies (see also Wolda 1978a). However, Takata (1961) reported that fecundity of *P. rapae* was depressed in hot weather. Dempster (1967) and Thomas (1974) observed that in temperate regions unfavourable seasons like cold or dull weather reduced the fecundity of the butterflies. It has also been shown that rainfall indirectly affects oviposition through host plants (Heslop *et al* 1964; Singer 1972; Seif 1981).

At the end of northeast monsoon, reproductive activity of *C. crocale* ceases and those adults emerged during the fag end of the monsoon (January) continue to survive till the commencement of the following southwest monsoon. These adults spend a reproductively inactive stage for a period of about 100–120 days. Using marking and recapture technique, we found that about 20 % of the adults were recaptured after 83 days during the non-monsoon period. Thus the survival of the adults during the non-monsoon period permitted in the initiation of oviposition during the next monsoon (southwest). Dunlop-Pianka (1979) reported the survival of actively reproducing adult Heliconius butterflies for about 6 months in the field.

Analysis of the data on life table/survivorship curve precisely showed that the early (egg and I instar) and the late stages (V instar and pupae) of *C. crocale* suffered heavy mortality during the entire study period of the 1980–81 and 1981–82 seasons. Key factor analysis (*k*) was followed to estimate age specific mortalities (Morris 1959; Varley and Gradewell 1960; Hayes 1981). These age specific mortalities (*k*) were added together to give the total generation mortality (*K*). Such analysis revealed that *k* values always remained high for egg and I instar as well as for V and pupal stages. Observation of Hayes (1981) for estimating the *k* values for the pierid butterfly *Colias alexandra*, was similar to the one observed in the present study. He reported that egg, diapausing larvae (final instar) and post diapausing larvae suffered the maximum mortality during the extended study period of 5 years. Working on the pierid butterfly *Anthocharis cardamines*, Courtney (1981) showed that these polyphagous larvae fed and survived on a variety of cruciferous food plants; irrespective of larval host plant, maximum mortality was obtained during egg, early and late larval instars. Egg mortality was due to cannibalism; late larvae and pupae of *A. cardamines* suffered a maximum of about 25–40% due to bacterial attack and 40% due to parasitism.

Wolda and Foster (1978) reported an outbreak of *Zunacetha annulata* in a neotropical forest on Barro Colorado Island during 1971–73 and showed that the outbreak was very effectively ended by fungus and/or either bacterial or viral disease. They have found that 90% of the pupae were killed by these factors and suggested the existence of density dependent factor which brought the numbers down whenever the larval population exceeded certain level.

Mathavan (1975) reported the incidence of a tachinid *Strumia convergens* on a population of the monarch butterfly *Danaus chrysippus* during 1973–74 and showed that about 15% of the pupae were killed by the parasite. Pandian and Delvi (1973) showed that about 11% of the grasshopper *P. pictus* population was destroyed by the sarcophagid fly *Blaesoxipha kaestneri*. Normally, both *D. chrysippus* and *P. pictus* are subjected to limited predator/parasite mortality, since they feed on the *Calotropis* plant and accumulate the carotenoid poison in their body (Brower 1969). Hence the percentage mortality due to parasite in *P. pictus* and *D. chrysippus* was minimal when compared to the data available in the literature. In the tropical moth *Achea janata*, Muthukrishnan (1980) observed about 70–80% of the population was reduced by the hymenopteran parasite *Microplitis ophiusa* (see also Nayar *et al* 1976). Presenting the life tables of caterpillar *Hemileuca oliviae*, Henson *et al* (1982) concluded that without parasitism, caterpillar density remained constant after a 70% minimum loss in the early stages. However, the occurrence of techinid parasite and predation decreased the density to an insignificant level.

C. crocale is a continuous breeder, and hence mortality per generation cannot be fixed for the population in the field. However, percentage of adults surviving in a season can be calculated. The calculation thus made revealed that, of the total eggs laid in the season about 1% or less than 1% successfully emerged as adults in the 1980–81 and 1981–82 seasons. McClure (1981) found that in the homopteran populations of *Fiorinia externa* and *Tsugaspidiotus tsugae*, the generation mortality ranged from 93–99%. Investigating the key mortality factors of *Heliothis armigera*, Bilapate (1981) reported a generation mortality of 99·2% and the major killing factors the population are the parasites and predators. A similar generation mortality was also observed in the pierid butterfly *C. alexandra*, which ranges from 97·1% in the year 1975 to 99·5% in the year 1978 (Hayes 1981). Latheef and Harcourt (1974) reported that the Colorado potato beetle *Leptinotarsa decemlineata* population suffered 99% mortality in the potato fields of eastern Ontario.

Many studies have shown that insects in the tropics are seasonal (Goel 1976; Wolda 1977, 1978b). Mostly, the fluctuation pattern is related to rainfall and wet and dry seasons. Working on the leaf hoppers in Panama, Wolda (1980) reported that in most species of Homopterans the maximum abundance peak was observed in the rainy season and the minimum in the dry season. This is very similar to the population of *C. crocale*. Moreover, seasonality of insect species is synchronized with the seasonal presence of its food (Wolda 1978b). Such a type of fluctuation related to rainfall is not uncommon in the tropics.

Wolda (1978a) clearly showed that such a type of fluctuation is found both in tropical and temperate zones. *C. crocale* population fluctuation is clearly influenced by the magnitude of rainfall during different months (dry and wet season) of the season. As the incidence of hymenopteran entomophagous parasite and bacteria fluctuates along with the *C. crocale* population it may be concluded that these factors influence *C. crocale* population considerably. Similar controlling factors for the population regulation have been reported (Wolda and Foster 1978; Harcourt 1971). According to Varley and Gradwell (1970) such fluctuation in the population should not be unexpected since mortality factor may not remain constant throughout the whole range of population density. Ehrlich (1965) also emphasized the fact that one factor which is extremely important in the dynamics of *E. editha* population is parasitism in addition to the microclimatic changes of the area.

The unfavourable environmental situations such as low rainfall, high temperature and piling up of heavy predation pressure plus maximum parasitic load resulted in the decline of *C. crocale* population to a minimum level. Decline in the density of the host resulted in the disappearance of the controlling factors. Ultimately, *C. crocale* adults survived during the non-seasonal period without much predation pressure and began to initiate the population build up with the commencement of the subsequent monsoon. The foregoing discussion clearly shows that though the environment controls the reproductive success of the butterfly, the survival and continuous propagation of the population mainly depend upon the load of parasitism and predation on the larval population.

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Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries

A H PARULEKAR, Z A ANSARI and B S INGOLE
National Institute of Oceanography, Dona Paula, Goa 403 004, India

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Abstract. Comparison of two clam beds and associated benthic fauna, in Mandovi and Cumbarjua canal estuarine system of Goa, severely affected by massive inputs of mining rejects, revealed that, in less than 10 years (1972–73 to 1982–83), high biotic variability induced by increasing environmental stress has caused irreversible ecosystem instability. Reduced dissolved oxygen concentration; high suspended solids and blanketing of bottom deposits by mining rejects, has resulted in more than 70 % reduction in clam production; near extinction of resident fauna and the appearance of a low diversity bottom fauna, comprising of tolerant but vagrant species. Ever increasing entry of mining rejects, which has reduced the healthy and highly productive estuarine environment of 1972–73, into an impoverished biotope, in less than 10 years, unless prevented will result in the total extinction of estuarine life in the near future.

Keywords. Mining rejects; clam bed; benthic fauna; environmental variability; faunal diversity; biomass.

1. Introduction

The coastline of Goa (between lat. 14°54′–15°48′N and long. 73°41′–74°20′E), measuring about 100 km in length, is characterized by estuaries of 7 rivers (figure 1). All these estuaries, providing sheltered habitat, harbour commercially important shellfish beds of clams, mussels and oysters, with an estimated yield of about 1000 tonnes/year (Parulekar and Qasim 1981). Being sessile and burrowing in habitat, the benthic or bottom dwelling organisms are the major casualities of any major environmental or man-made changes (Heip 1980).

A major contribution to the economy of Goa is from the export of over 10 million tonnes/year of iron and manganese ores from Marmugao port. All the ore extraction operations are carried out in the vicinity of river systems and, further the transport of ore for export shipment is done by mechanized barges, plying solely through the inland waterways of Mandovi-Cumbarjua canal—Zuari estuarine system (figure 1). Extraction of one tonne of export quality ore, generates about 1·5 tonnes of mining rejects and over the years, more than 300 million metric tonnes of rejects are estimated to have accumulated in the mining belt of Goa (Anonymous 1981). Due to heavy southwest monsoon rains, (average rainfall varying from 3 meters/year in the coastal areas to 3·75 meters/year in the hilly regions, where the mining areas are located) these mining rejects get eroded due to flooding, and are transported downstream the rivers, when about 11 % of the mining rejects settle in the sediment at 0–5 % salinity and about 21 % are lost to bottom deposits at 15–20 % salinity (Anonymous 1981). Thus, a substantial part of these mining rejects, ultimately settle in estuarine benthic

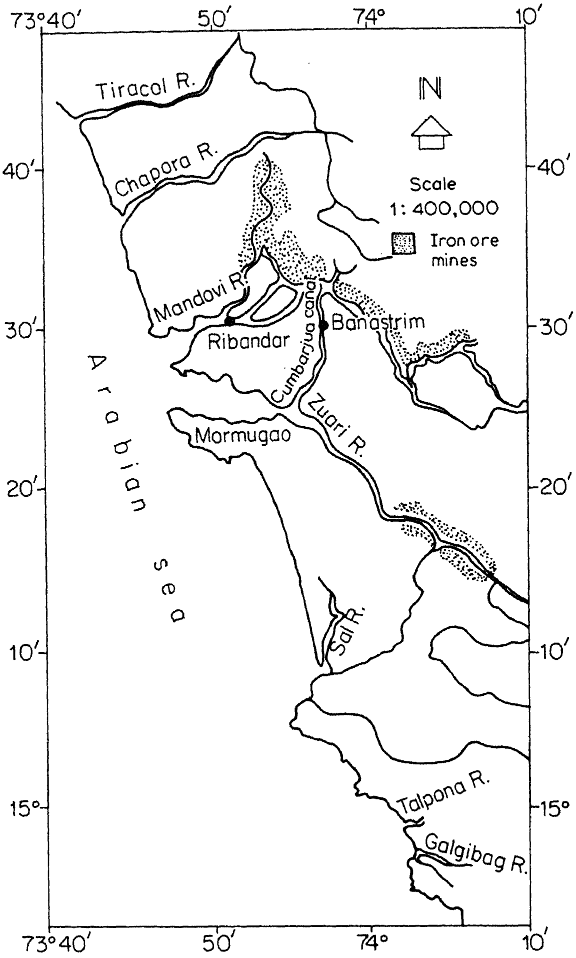

Figure 1. Map of Goa showing the mining area and the study area—Ribandar and Banastrim.

environment, having an extensive mesohaline (5–18%) and polyhaline (18–30%) salinity regime (Parulekar and Dwivedi 1974).

Earlier in 1972–73, a detailed study on the ecology of clam beds and associated fauna, at Ribandar, in Mandovi River and Banastrim, in Cumbarjua Canal, was carried out (Parulekar *et al* 1973). Recently, i.e. in 1982–83, the clam beds at Ribandar and Banastrim were again studied and the changes observed in environmental and biotic components are presented and discussed in relation to mining activities.

2. Material and methods

Clams, *Meretrix casta* (Chemnitz), were collected at fortnightly interval (1982–83) from the subtidal clam beds at Ribandar and Banastrim (figure 1). Both the sites were sampled on the same day and during the same tidal phase, by using a Van Veen grab, having a substrate coverage of $0{\cdot}1\ m^2$, upto a penetration depth of 10 cm. Samples were collected in replicate of 5 and the mean value of biomass (wet weight) and population density was estimated.

After removing clams, the remaining sediments were sieved through a 0·5 mm mesh screen and the fauna retained in the sieve was preserved in 5% seawater—formaline—Rose Bengal solution, for observations on the distribution, abundance and species composition of benthic fauna associated with the clam bed. Environmental data on temperature, salinity, dissolved oxygen and suspended solids in the bottom waters, about 30 cm above the clam bed, was collected during each sampling trip. Temperature and salinity were recorded directly on a NIO (India) design Temperature-Salinity bridge. Dissolved oxygen was estimated by winkler method (Strickland and Parsons 1972) and the amount of suspended solids, was determined by filtering one litre of near bottom water through a pre-weighed Whatman GF/A filter paper, which was dried at 105°C and reweighed. Organic carbon in sediments was estimated by the method of El Wakeel and Riley (1956).

As biomass and population density counts are usually found to be skewed in benthic surveys, a log transformation [$\log (x+1)$] of biotic parameters was carried out, for statistical treatment (Gage and Tett 1973). Similarly, the interpretation of data, pertaining to two different periods of observations i.e. 1972–73 and 1982–83, were tested for the magnitude of interaction and significance of relationship, by calculating the correlation coefficient (r) and testing it by t-test ($P < 0{\cdot}05$). Data on environmental and biotic variables, is presented (range, mean and 95% confidence limit) by Dice-Lerass diagrams. Species diversity was estimated by using the index of diversity (Margalef 1958).

3. Results and discussion

3.1 *Environmental features*

The average depth at the sampling sites varied from 2·2–3·5 m (Ribandar) to 2·6–3·3 m (Banastrim) and the variations were relevant to tidal phase and seasonal flooding due to land run-off. As compared to 1972–73, no major changes, in depth over the clam beds, were observed (Parulekar *et al* 1980).

Bottom water temperatures (0·3 m above the clam bed) displayed a comparable trend in range, mean and variability, both in 1972–73 and 1982–83. Though no major changes were observed in 1982–83, the maximum temperature, both at Ribandar and Banastrim, was almost 4°C higher than in 1972–73 (figure 2).

Range of salinity values at Ribandar in 1982–83 was relatively small and of lower magnitude than in 1972–73, and consequently the observed variability was more intense in 1972–73 than in 1982–83 (figure 2). In contrast, the range, mean and magnitude of variability, in salinity values at Banastrim, was comparable in both the periods of observations.

Dissolved oxygen concentration in 1982–83 showed major changes in range, mean and magnitude of variability, at both the sampling sites (figure 2). While the range and mean values, in 1972–73 at Ribandar and Banastrim, were uniformly high but with small variability, by 1982–83, the range and mean values though of low magnitude, showed relatively high variability, especially at Ribandar. Mean values of 2·69 ml/l

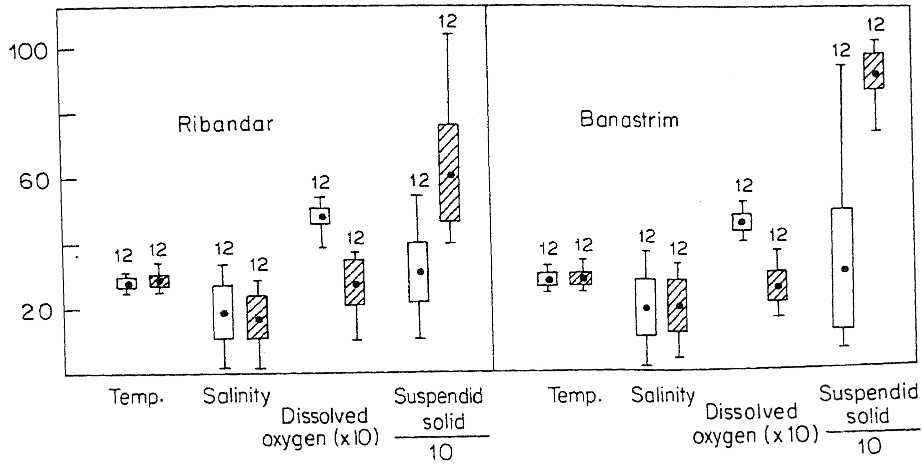

Figure 2. Range, mean and variability in environmental factors at Ribandar and Banastrim in 1972–73 and 1982–83 (stripped).

(Ribandar) and 2·55 ml/l (Banastrim) in 1982–83 observations, clearly indicate that an overall 66–67 % depletion in dissolved oxygen, has taken place in the clam bed environment, in a span of 10 years (1972–73 to 1982–83).

Major changes in the suspended solid content of the bottom waters overlying the clam bed, were evident (figure 2). At Ribandar, the range, mean and variability was higher in 1982–83 than in 1972–73. In contrast, at Banastrim, while the range of values and the magnitude of variability, was rather wide in 1972–73, the mean value as well as the range, was many times more, but with less variability in 1982–83. It suggests that, while the suspended solids in the water column at both the clam beds, have increased considerably within 10 years, the relative increase at Banastrim is more consistent and of higher magnitude than at Ribandar. This is attributed to the flow characteristics wherein flow of water is from Zuari estuary into the Cumbarjua canal (Das *et al* 1972).

Retrogressive changes, in the composition of bottom deposits, were observed. In 1971–73, the type of bottom deposits, at Ribandar and Banastrim, were characterized as sandy to muddy sand, with mean grain size of 0·5–0·25 mm and 0·25–0·125 mm, respectively (Parulekar *et al* 1980). However, in 1982–83 survey, the sandy and the muddy sand type of bottom deposits, were observed to be covered with red clay and further blanketed by cobble and pebbles of lateritic origin (figure 3).

Organic carbon content of bottom deposits in 1972–73 varied from 0·11–2·55 % with a mean value of 0·78 % at Ribandar and from 0·24–1·8 %, with a mean value of 1·0 % at Banastrim (Parulekar *et al* 1980). Inspite of a marginal increase in annual mean of 0·04 % at Ribandar and 0·02 % at Banastrim, the range (0·1–2·72 %) and the magnitude of variability in 1982–83, was almost similar to that in 1972–73. It means that there was no substantial increase in the organic enrichment of clam habitat, in the last 10 years.

3.2 *Biotic characteristics*

The clam biomass (figure 4) in two different periods of observations, showed overall decrease in range, mean and variability. At Ribandar, in 1972–73, the biomass values,

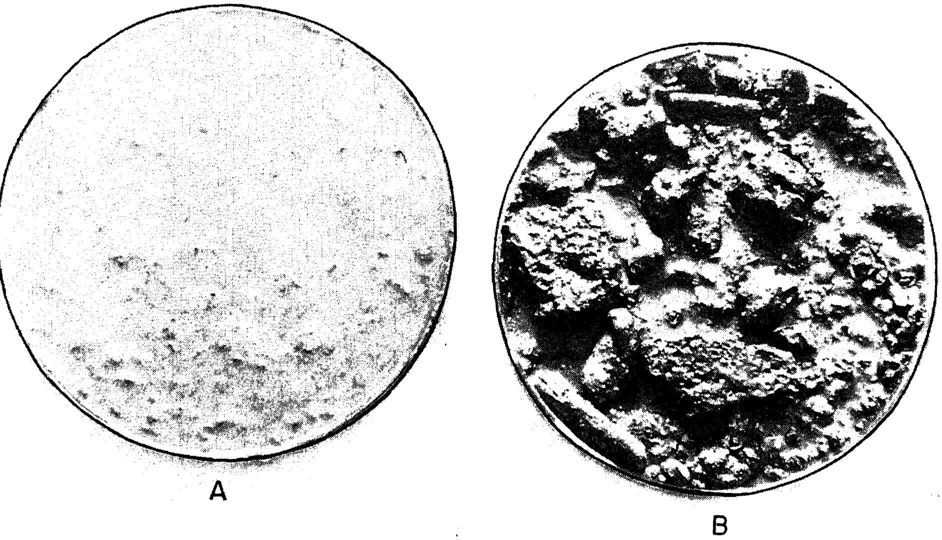

Figure 3. Bottom deposits at clam bed. **A.** Sandy-mud type of bottom, as occurred in 1972–73. **B.** Sandy-mud bottom deposits covered by cobble and pebbles as in 1982–83.

vhile varying between 133·9 and 2381·3 g^{-2} ($\bar{x}$ = 538·4 g^{-2}), displayed a high nagnitude of variability. By 1982–83, not only the range and mean value (0·1–38 g^{-2}; $\bar{x}$ = 10 g^{-2}) but also the variability declined considerably. At Banastrim, almost similar lepletory trend was observed. The high range and high mean value (149·2–3060·5 g^{-2}; $\bar{x}$ = 779·7 g^{-2}) as observed in 1972–73 decreased, both in range (4·4–392·2 g^{-2}) and nean value (151 g^{-2}) in 1982–83. Consequently, the high magnitude of variability in lam biomass, as observed in 1972–73, reduced to a low level in 1982–83 (figure 4).

Population density of clams, in space and time, revealed (figure 4) that from the igh range and high mean value of 150–2075/m^2 ($\bar{x}$ = 893/m^2) and 450–22012/m^2 ($\bar{x}$ = 3969/m^2) in 1972–73 at Ribandar and Banastrim, respectively, the observed decline in 1982–83, ranged from 3–98/m^2 ($\bar{x}$ = 36/m^2) at Ribandar to 90–720/m^2 ($\bar{x}$ = 360/m^2) t Banastrim. Magnitude of variability, which was very high in 1972–73 at Ribandar, eached very low level in 1982–83, with range and mean value at their lowest. Total bsence of clams, in the month of March, November and December at Ribandar and in he month of July at Banastrim, was also observed in 1982–83.

Benthic standing crop, estimated as benthic biomass, varying from 0·1–228·9 g^{-2} ($\bar{x}$ = 56·8 g^{-2}), but with moderately high variability (figure 4), as observed in 1972–73 at ibandar, declined to 0·5–190·6 g^{-2} ($\bar{x}$ = 23·8 g^{-2}) with low variability in 1982–83. imilarly, at Banastrim, the high range (33·1–876·9 g^{-2}) with relatively high variability nd high mean value of 339·6 g^{-2} in 1972–73, while displaying almost 50 % reduction, oth in range (0·8–331·6 g^{-2}) and mean value (153·2 g^{-2}) in 1982–83, had comparable nagnitude of variability (figure 4) as in 1972–73.

Population density of benthic fauna at Ribandar, which declined from 648–3900/m^2 $\bar{x}$ = 1864 m^2) in 1972–73 to 200–1500/m^2 ($\bar{x}$ = 831/m^2) in 1982–83, displayed omparable magnitude of variability during both the periods of observations (figure 4).

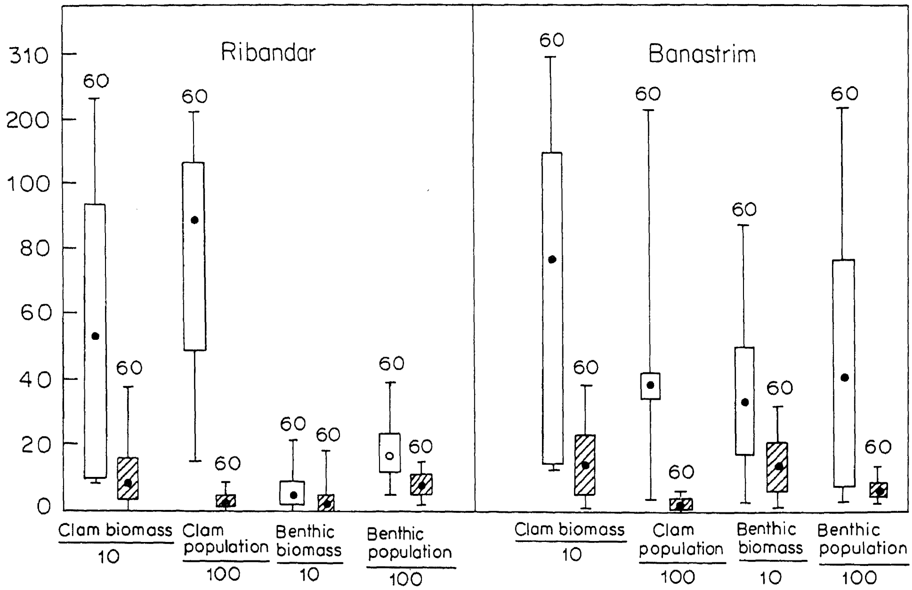

Figure 4. Range, mean and variability in biotic characteristics at Ribandar and Banastrim in 1972–73 and 1982–83 (stripped).

In contrast, at Banastrim, the population density, which exhibited high range (875–22350/m^2), high mean value (4274/m^2) and high magnitude of variability in 1972–73, decreased considerably in range (50–1575/m^2), mean (750/m^2) and variability in 1982–83 (figure 4).

Faunal composition at two sampling sites during the two periods of observations, indicated (table 1) that while the prevalence of a particular faunal taxa underwent minor changes, within a span of 10 years, there were noticeable changes, both qualitative and quantitative, in the presence or absence of species. While the number of species at Ribandar increased from 24 in 1972–73 to 30 in 1982–83, the number of species at Banastrim, decreased from 15 in 1972–73 to 12 in 1982–83. The total number of species at Ribandar and Banastrim, was 46 and 23, respectively. The break-up of 8 common species at Ribandar was, 6 species of polychaetes (*Nereis capensis*, *N. mirabilis*, *Perinereis nuntia*, *Prionspio cirrifera*, *Diopatra neapolitana* and *Marphysa sanguinea*) and a species each of pelecypoda (*Meretrix casta*) and amphipoda (*Corophium* sp.). At Banastrim, the 4 common species were 2 polychaete species (*Lepidonotus carinulatus* and *Diopatra neapolitana*) and a species each of gastropoda (*Cerithidea fluviatilis*) and pelecypoda (*Meretrix casta*).

Changes in species composition, in a span of 10 years, was further investigated by studying the composition of feeding types in the fauna (table 1). At Ribandar clam bed, the benthic fauna in 1972–73, was characterized by the dominance of filter feeding and detritus feeding species, basically infaunal in habitat. It reflects on the availability of the food material, both in the overlying water column as also on the surface of the bottom deposits. However, by 1982–83, with alarming increase in the amount of inorganic suspended solids, the food supply was drastically cut and accordingly, the scavenger species, epibenthic or migratory in habitat, attained the dominance. Similarly, at

Table 1. Faunal composition, number of species and feeding types in associated fauna of clam beds at Ribandar and Banastrim in 1972–73 and 1982–83.

Faunal taxa	Ribandar (No. of sp) 1972–73	Ribandar (No. of sp) 1982–83	Banastrim (No. of sp) 1972–73	Banastrim (No. of sp) 1982–83
Foraminifera	1	—	1	—
Actiniaria	—	2	—	—
Polychaeta	12	12	6	5
Gastropoda	1	3	2	1
Pelecypoda	4	4	2	1
Cirripedia	1	—	—	—
Amphipoda	2	4	1	1
Alphidea	—	1	—	1
Decapoda	3	3	3	2
Teleostei	—	1	—	1
No. of species	24	30	15	12
No. of common species	8		4	
Total No. of species	46		23	

Locality	Year	Feeding Types—Percentage composition Total No. of species	Filter feeders	Detritus feeders	Carnivores	Scavengers
Ribandar	1972–73	24	33·5	29·0	12·5	25·0
	1982–83	30	13·3	13·3	6·6	66·8
Banastrim	1972–73	15	26·7	33·3	20·0	20·0
	1982–83	12	8·3	8·3	-	83·4

Banastrim, the dominance of filter feeders and detritus feeders in 1972–73, was replaced by the overwhelming dominance (83·4 %) of scavenger species in 1982–83.

Species diversity index describes the effect on the fauna by changes in the environment. The index of diversity (table 2), as assessed from the temporal variations in the distribution and abundance of species, at Ribandar and Banastrim, during 1972–73 and 1982–83, revealed that, in accordance with the high magnitude of variability in environmental factors, there was higher species diversity in 1972–73 than in 1982–83, at Ribandar. At Banastrim, while the index of diversity was higher in 1972–73 than in 1982–83, the magnitude of variability, surprisingly, was more intense in 1982–83 than in 1972–73. It indicates that the epibenthic or vagrant species are not present throughout the year. Number of species and the number of specimens, at both the sampling sites and in two different periods of observations, showed a declining trend, during the different months of monsoon season (June–September), apparently because of lowering of salinity due to heavy land run-off; and consequently, the species diversity, attained its lowest, during June–September period, depending upon the year-to-year variations in rainfall and flooding. As assessed from the annual mean (table 2), it may be inferred that the faunal diversity, over the years, decreased considerably, from 5·4 ± 0·91 (1972–73) to 4·1 ± 0·61 (1982–83) and from 2·8 ± 0·43 (1972–73) to 1·5 ± 0·53 (1982–83), at Ribandar and Banastrim, respectively. The changes in diversity can be correlated to simultaneous changes in the suspended solids and sediment composition which made benthic environment unsuitable for colonization by many sensitive and rare species and were duly replaced by a few tolerant species. Such striking decline in species diversity, is a reflection of environmental perturbation and the response of biota, over the span of 10 years (Lie 1968).

The magnitude of interaction between environmental and biotic variables, determines the quality and quantity of the biota and, therefore the relationship between them was attempted (table 3). The correlation coefficient (r), which measures the magnitude of interaction, was tested by t-test ($p<0·05$). The significant correlation, observed between abiotic and biotic variables was as summarized below:

	1972–73	1982–83
Ribandar	Salinity-benthic biomass Salinity-No. of species Suspended solids-benthic biomass Suspended solids-benthic population density Suspended solids-No. of species.	Salinity-benthic population density Salinity-No. of species Suspended solids-benthic biomass Suspended solids-benthic population density Suspended solids-No. of species
Banastrim	Salinity-clam biomass Salinity-No. of species Suspended solids-benthic biomass Suspended solids-benthic population density	Dissolved oxygen-No. of species

These relationships indicated that salinity-especially during the monsoon season (June–September) and suspended solids, both were related to the occurrence of number of species, at Ribandar and Banastrim, in 1972–73. Suspended solids, in the overlying water column above the clam beds, showed significant relationship with benthic biomass, benthic population density and number of species at Ribandar, both in

Table 2. Number of species, population density and index of diversity in associated fauna of clam beds at Ribandar and Banastrim in 1972–73 and 1982–83.

	Ribandar						Banastrim					
	1972–73			1982–83			1972–73			1982–83		
Month	Species	Population density	Index of diversity	Species	Population density	Index of diversity	Species	Population density	Index of diversity	Species	Population density	Index of diversity
Jan.	23	1200	7·1	12	1075	3·6	13	4987	3·2	6	1300	1·6
Feb.	21	1780	6·1	11	1475	3·1	14	1538	4·0	6	225	2·1
Mar.	22	1300	6·7	17	1025	5·3	12	1200	3·5	8	600	2·5
Apr.	24	1975	6·9	16	725	5·2	10	2887	2·6	4	300	1·2
May	21	2225	5·9	17	1125	5·2	12	22350	2·5	5	1225	1·2
June	17	725	5·6	10	200	3·9	12	875	3·7	7	650	2·1
July	9	648	2·8	11	425	3·8	7	1675	1·8	3	50	1·1
Aug.	10	2750	2·6	7	250	2·5	8	3112	2·0	2	350	0·4
Sept.	16	1325	4·8	9	475	2·9	11	2812	2·9	2	875	0·3
Oct.	19	2687	5·2	16	1125	4·9	10	2700	2·6	4	1575	3·2
Nov.	22	3900	5·8	15	1500	4·4	9	1800	2·4	5	1050	1·3
Dec.	22	1855	6·4	13	575	4·3	14	5362	3·4	7	800	2·0
Mean			5·4			4·1			2·8			1·5
95% confidence limit ±			0·91			0·61			0·43			0·53

Table 3. Magnitude of interaction between environmental parameters and biotic components at Ribandar and Banastrim in 1972–73 and 1982–83.

		Banastrim								
		Temperature (°C)	Salinity (‰)	Dissolved oxygen (ml/l)	Suspended solids (mg/l)	Clam biomass (g^{-2})	Clam population (n/m^2)	Benthic biomass (g^{-2})	Benthic population density (n/m^2)	No. of species
Temperature (°C)	1972–73	1·0	0·41	0·29	−0·44	−0·14	−0·17	0·02	0·18	−0·10
	1982–83	1·0	0·60^a	−0·48	−0·44	−0·12	0·24	−0·04	0·33	0·06
Salinity (‰)	1972–73	0·43	1·00	−0·55^a	−0·83^a	−0·73^a	0·21	0·15	0·18	0·62^a
	1982–83	0·43	1·00	−0·61	−0·73^a	0·01	0·33	−0·02	0·33	0·42
Dissolved oxygen (ml/l)	1972–73	−0·12	−0·10	1·00	0·33	0·47	−0·15	−0·11	−0·29	−0·29
	1982–83	−0·15	−0·58^a	1·00	0·81	0·22	−0·12	0·23	−0·09	−0·60^a
Suspended solids (mg/l)	1972–73	−0·29	−0·58^a	0·74^a	1·00	0·39	−0·38	0·54^a	−0·55^a	−0·70^a
	1982–83	−0·29	−0·91^a	0·55^a	1·00	0·04	−0·25	0·05	−0·23	−0·42

Ribandar										
Clam biomass (g^{-2})	1972–73	0·22	−0·33	−0·16	0·21	1·00	0·19	−0·09	0·25	−0·26
	1982–83	0·11	0·16	0·08	−0·19	1·00	0·81	0·96^{a}	0·78^{a}	−0·02
Clam population (n/m^{2})	1972–73	−0·47	−0·44	−0·27	−0·15	0·32	1·00	0·43	0·68^{a}	0·37
	1982–83	0·31	−0·02	−0·14	0·04	0·59^{a}	1·00	0·81	0·92^{a}	0·29
Benthic biomass (g^{-2})	1972–73	−0·22	0·62^{a}	−0·35	−0·61^{a}	−0·47	−0·42	1·00	−0·54^{a}	0·30
	1982–83	−0·09	0·46	−0·08	−0·53^{a}	0·81	0·28	1·00	0·83^{a}	−0·01
Benthic population density (n/m^{2})	1972–73	0·31	0·33	−0·38	−0·57^{a}	−0·22	0·11	0·74^{a}	1·00	0·49
	1982–83	−0·24	0·53^{a}	−0·01	−0·53^{a}	−0·16	−0·46	0·26	1·00	0·27
No. of species	1972–73	0·31	0·87^{a}	−0·32	−0·69^{a}	−0·53^{a}	−0·43	0·71^{a}	0·32	1·00
	1982–83	0·38	0·75^{a}	−0·32	−0·63^{a}	−0·23	−0·24	−0·03	0·52	1·00

$^{a}P < 0·05$.

1972–73 and 1982–83. At Banastrim, in 1972–73, the suspended solids displayed a significant relationship with benthic biomass and benthic population density, as against the only significant relationship between dissolved oxygen and number of species, in 1982–83. From table 3, it may be deduced that at Banastrim, in a span of 10 years, between 1972–73 and 1982–83, severe destruction accompanied by complete instability of the clam bed ecosystem has taken place, and consequently, the magnitude of interaction between environmental variables and biotic variables has become insignificant. At Ribandar, on the otherhand, the interaction, especially between the suspended solids and the biotic variables of the clam bed ecosystem, is significantly maintained even in 1982–83. It indicates that while the assimilative capacity of clam bed ecosystem at Ribandar, as evidenced from the considerable impoverishment of biota, is on decline, the overall stability is still being maintained, though at a much lower level than in 1972–73. However, with the continuing deterioration in the environmental quality, it is only a matter of time, before the total disintegration of assimilative capacity and instability of clam bed ecosystem, takes place.

Field measurement of biological impact of pollutants, as in the present study, has a significant role in the management and conservation of sensitive but highly productive estuarine environment. The monitoring of the biological effects of marine pollution, can be carried out at any level of biotic organization, from the ecosystem, community, population density, organismic down to the cellular levels, by undertaking comparative studies on distribution, abundance and structure of a population (Widdows 1983). Measurement at community and population structure level provides an assessment of impact of the long term consequences of high levels of chronic pollution. Though, quite a few studies on the long term effects of pollutants on estuarine and marine biota have been reported (Bayne 1978; Parulekar *et al* 1985), very little (Norris *et al* 1982) is known about the long term effects of onshore mining on the biota of coastal ecosystems.

Benthic macroinvertebrates, as a group of organisms, having infaunal and/or epifaunal habitat, are very sensitive to changes in the environment, as they integrate the combined effect of many stresses through changes in their ecology, morphology and physiology (Ketchum 1973). As a group, they are amenable to numerical quantification and hence, they are ideally suited for assessing the impact of pollution in estuarine and marine environment (McIntyre and Pearce 1980).

Amongst the multitude of benthic organisms, bivalve molluscs, like clams, mussels and oysters, have widely been used as sentinel organisms, for assessing and monitoring the chemical and biological effects of marine pollution (Hung *et al* 1975). Besides, being commercially important and also the dominant members of coastal and estuarine ecosystem, the bivalves being sessile in habit, passively experience the varying conditions in the overlying water column. As they pump large quantity of water, for feeding and respiration, they accumulate contaminants and are reported to be responsive to many of the environmental pollutants, without showing a prolonged stress (Widdows *et al* 1980).

Environmental and biological parameters, studied during the course of the present study, are essential to correlate the frequency of occurrence, faunal composition, standing crop and community structure of the clam bed ecosystem, in relation to changes in the physical, chemical and geological characteristics, over a period of 10 years, from 1972–73 to 1982–83. Relative abundance and changes in spatial and temporal distribution of clams and the associated benthic fauna, gives a good indication of the magnitude of environmental changes, principally, brought about by the entry of mining rejects, on an everincreasing scale, in the Mandovi-Cumbarjua canal-Zuari estuarine system of Goa.

Mining activities are about double as much along the Mandovi river than along the Zuari river (Anonymous 1979) and accordingly, the ecosystems in Mandovi estuary, receive the maximum adversity, due to mining rejects. Kamat and Sankaranarayana (1974) observed high value of 40·3 mg/l of particulate iron in the nearbottom water at Ribandar as compared to 6·6 mg/l at Banastrim and further reported that about 70,000 tonnes of iron, in particulate form, gets annually deposited in Mandovi river. Based on a 1977–78 study, significantly high concentration of iron and manganese in the sediments of Mandovi and Zuari river, with moderate concentrations in the interconnecting Cumbarjua canal have been reported (Anonymous 1979).

The fate, and the probable effects on the benthic fauna, of a metal, from particulate to dissolved state, in interstitial waters within the sediments, vary greatly (Krom and Sholkovitz 1978). For, iron and manganese, there is a significant release from the interstitial waters in response to the degradation of organic matter (Lu and Chen 1977) and further conversion to dissolved state in the overlying oxygenated water and dispersal by physical forces of currents and tides (Bertine 1982; Gideiri 1984). While explaining the causes for the considerable depletion of dissolved oxygen (66–67 %), as observed in the present study, also provides a clue for the non–toxic effects of mining rejects to the benthic fauna.

Besides the overall decrease in the dissolved oxygen concentration, other obvious reasons for the decline in the clam resources are due to the immense increase in the quantity of suspended solids and structural deformation of bottom deposits (figure 3).

An ideal habitat for burrowing organisms, like clams is of soft sediments, a combination of sand and silt. Any change to red clay, cobbles and pebbles, as observed in the present study, will deter the settlement and growth of suspension feeders, like clams and a majority of infaunal benthic invertebrates. Even if the pelagic larvae of clams, succeed in settling on the altered bottom deposits, then the observed, alarming increase in the suspended solids, principally of inorganic origin, while reducing the availability of conventional suspended organic matter, will also result in the choking of filtration apparatus, with possible mortality of the infaunal assemblages. As a consequence, the dominant filter feeding and detritus feeding species of 1972–73 have been replaced, in reduced population density and biomass, by scavenger or migrant species, comprising of opportunistic organisms like amphipods, alpheids and errant polychaetes (table 1). It gave rise to the observed low faunal diversity, due to the replacement of multispecies infaunal assemblages of resident fauna of 1972–73 by few tolerant, but epifaunal and migrant species of 1982–83.

Evidently, the red clay particles, both in suspension as also settled on the bottom deposits alongwith the lateritic cobble and pebbles strewn in the clam beds, are of terrigenous origin, and have entered the estuarine environment through land run-off from the mining rejects and due to spilling, handling mishaps and washings from mechanized barges, transporting iron ores, from mine head to the shipment point. Such massive inorganic inputs, estimated to be about 70,000 tonnes of iron, in particulate form, getting deposited annually in the Mandovi river (Kamat and Sankaranarayana 1974), has resulted in the considerable reduction of the assimilative capacity of the clam bed ecosystem at Ribandar and serious ecosystem instability at Banastrim clam bed. These deleterious man-made changes though gradual are recurring in nature and unless abated, the total extinction of estuarine living resources, is evident.

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Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts

R V KRISHNAMOORTHY and S N VAJRANABHAIAH*

Department of Zoology and *Department of Crop Physiology, University of Agricultural Sciences, Bangalore 560 065, India

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Abstract. Biochemical analyses and auxin and cytokinin bioassays were performed to test the biological activity of wormcasts. Both cellulose paper pulp and soil casts of *Lampito mauritii* were rich in ammonia, urea, organic carbon content, organic matter, soluble phosphorus and ionic potassium levels. The total nitrogen content of the soil remained unaffected by worm activations. The casts of *Lampito mauritii*, *Pheretima elongata*, *Pontoscolex corethrurus* and *Ocnerodrilus occidentalis* had greater urea levels relative to ammonia levels in contrast to those of *Drawida barwelli*, *Octochaetoides beatrix* and *Perionyx excavatus*. Worm activations of the leaf compost amended-soils by these species reduced the total phenol levels to varying degree depending on the species examined. Aging and exposures to light reduced the activity levels of these plant growth promotors in the casts of *L. mauritii*. A positive correlation between the worm density at the site of soil sampling and the plant growth promotor levels in the samples was obtained. The origin of these promotors from the gut microflora of the worm and their subsequent release into the environment were discussed.

Keywords. Tropical earthworms; earthworm casts; composition of wormcasts; plant growth promotors in wormcasts; biological activity of wormcasts.

1. Introduction

In tropical biotopes the wormcast production ranges from 13–278 tons ha^{-1} y^{-1}, (Ljungstrom and Reinecke 1969; Lavelle 1978; Dash and Patra 1979; Krishnamoorthy 1985). The casts can be an excellent soil adjuvant besides their value as biofertilizer (Tomlin 1983). The casts maintain more nutritive materials like oxidizable carbon, nitrogen Ca, Mg, K, Na and P than the soil (Dash and Patra 1979; Kale and Krishnamoorthy 1981). The conversion of soil organic matter into vermicompost or casts reduces the total nitrogen content of the ingested material. The NPK value of the casts is always lower than that of an acceptable standard fertilizer (Tomlin 1983). Several trials performed in the past have indicated (Keshavamurthy 1978; Reinecke and Visser 1980) that the vermicompost or wormcasts could promote the lush growth of plants. It is believed that this property may be due to the occurrence of plant growth promotors in the casts which might be a resultant of interactions existing between the microflora and the earthworm's gut (Satchell 1983). Hence, an intensive study of wormcast composition and the levels of plant growth promotors in the casts were made.

2. Material and methods

2.1 *Collection of worms and cultures*

The worms were collected from woodland and grassland areas of Bannerghatta, about 25 km south of Bangalore. They were stocked in culture pots with garden soil mixed with activated dung manure (3 : 1 proportion) as food. Five hundred adult (clitellate) worms of each species were left in a plastic box by 15 × 20 cm square by 10 cm height (1:2 w/w) containing 1 kg decomposing leaf litter compost-amended garden soil. The floor of each box was made of bolting cloth with 35 mesh pores as described earlier (Satchell and Martin 1984). After introducing the worms, the boxes were covered with a black cloth to prevent interference of light on worm activity. Prior to introduction, the worms were allowed to gut-void for 8 hr into a clean Petri dish and the excrements were separated. A similar box containing the soil but without worms served as control. After 3 days of worm activation at 25 ± 2°C the worms were hand sorted and separated from their castings and soil in the box. The contents of the control box and worm activated box were mixed and used for analysis.

Cultures of each species were left for one week for one hundred gut-voided worms to work through finely shredded paper pulp weighing 250 g taken in a plastic box as described above. A box containing the paper pulp without worms served as control. One gram of phytin (calcium inositol hexaphosphate) was mixed to each of 40 g of paper pulp. Samples of fresh faecal material and of the control medium equivalent to 1 g dw were then collected for the study.

2.2 *Worm densities and soil samples*

Worm densities in different biotopes around Bangalore were quantified by quadrat sampling technique (Lewis and Tayler 1968). Soil samples of the same biotopes were collected at random from sub soil layer (5–10 cm below the surface) and used for assays.

2.3 *Extraction of plant growth promotor substances*

One kg castings, pooled from 4 to 5 paper pulp culture sets or soil casts collected from the field afresh, were boiled in 2 l distilled water on a water bath for 30 min and filtered through a cheese cloth. Dilute phosphoric (1 : 10 v/v) acid was added drop by drop until the pH of the filtrate dropped down to 3·00. Then 200 ml ethyl acetate was mixed. The ethyl acetate fraction was separated by centrifugation and then removed by aspiration. The debris was extracted with ethylacetate in the same way and the fractions were combined and neutralised by partitioning with 5% aqueous $NaCO_3$ in a separating funnel. The ethyl acetate fraction was concentrated under low pressure and the concentrate was chromatographed.

2.4 *Thin layer chromatography of growth promotors*

Silicated thin layer chromatograms of 50 μl ethyl acetate fractions were approximately run for 45 min under red light in a darkroom to check the purity of auxin and cytokinins. Marker plots were made with indol acetic acid and benzyl adenine (both obtained from Sigma Co.). The plates were developed in isopropanol-water-conc. ammonia (9 : 1 : 1 v/v) for 45 min and the spots were visualised by charring at 105°C after spraying 6N H_2SO_4. The identified regions on the uncharred and dried plates were scrapped and eluted into 5 ml distilled water acidified with a drop of (1 : 1000 v/v) dilute HCl just before their use for bioassays. Otherwise they were preserved dry on the plate under darkness.

2.5 *Chemical analyses*

The wormcasts and soil samples were analysed for the organic constituents according to the methods presented in table 1.

2.6 *Corrections for the synergistic effects of potassium in the sample bioassays*

The potassium levels of both the field and purified samples were determined flame photometrically. Since it is known that the endogenous potassium levels of the sample, synergistically affect the bioassays (Ezekiel *et al* 1978; Green and Muir 1979), simultaneous bioassays were run for the corresponding endogenous potassium levels. Thus the promotor level in samples obtained through bioassays were corrected after measuring the cotyledon responses for the observed potassium level of the sample.

2.7 *Growth promotion of wheat seedlings*

The shoot elongation and shoot wet weight biomass of the wheat seedlings (*Triticum aestivum* Linn.) in different soil amendments with wormcasts were measured according to the methods described by Arditti and Dunn (1969).

Table 1. Methods followed for the studies on the composition of wormcasts and soils.

Constituent estimated	Method followed	Author(s) referred
Nitrogen	Macro-kjeldahl	Allen *et al* (1974)
Carbon	Dry ashing	Allen *et al* (1974)
Phosphorus (inorganic)	Molybdovanadate-colorimetric	Wilde *et al* (1972)
Oxidizable organic matter	Walkley and Black-modified titrimetric	Jackson (1967)
Exchangeable potassium	Flame photometry	Mehlich (1956)
Ammonia	Colorimetric (after Nesslerization)	Oser (1965)
Urea	Diacetyl monoxime-colorimetric	Oser (1965)
Total phenols	Colorimetric	Hartenstein and Hartenstein (1981)
Auxins	Wheat coleoptile bioassay	Setty and Wheeler (1968)
Cytokinins	Cucumber cotyledon bioassay	Udayakumar and Sastry (1973)

3. Results

The casts of *Lampito mauritii* Kinberg were rich in ammonia, urea, oxidisable organic matter, exchangeable potassium and phosphorus, total nitrogen, and plant growth promoting substances (table 2). Phenol and organic carbon contents were lesser in the casts than in surrounding soils. The control (wormless) soil had a C/N ratio of 5·26, and this was lowered due to casting activity of worms to about 0·82. Similarly, the control soil had a urea/ammonia (U/A) ratio of 0·87, which was altered to 3·34 due to worm activities, indicating that the urea content of the soil increased. The total phenol content of the soil was reduced significantly by the casting activity. Both cytokinin and auxin activities were increased in soils by the worm activities (table 2).

The activities of individual species brought significant changes in the U/A ratios, phenol levels and plant growth promotor substances in the leaf compost-amended soils (table 3). Compared to wormless control, the worm activated soils showed a significant reduction in the phenol levels. Initially, the wormless control soil had a 0·56 ± 0·03 mg phenols per 100 g dw soil. This level was brought down to 17–55 % by the activities of different earthworm species (table 3). The activities of *Perionyx excavatus* E. Perrier reduced the phenol levels more than those of any other species compared; and of *Octochaetoides beatrix* Beddard reduced to a poorer extent. Species differed with reference to the urea and ammonia contents of their castings (table 3). The castings of *L. mauritii* Kinberg had a higher U/A ratio than those of other species examined; and those of *O. beatrix* Beddard had the lowest ratio (table 3). Besides, urea, ammonia and phenols, the wormcasts also contained plant growth promotors such as cytokinins and auxins (table 2). The rates of production of these substances were evaluated by estimating the total excretory output of them through casts in a day. Such an analysis (table 3) indicated that *Pheretima elongata* E. Perrier released greater quantities of them through casts than any other species examined. *Drawida barwelli* Beddard and *O. beatrix* Beddard released them at the lowest rate. The release of these promotors through castings varied in various species in the following descending order: *Pheretima elongata* E. Perrier — *Perionyx excatatus* E. Perrier — *Pontoscolex corethrurus* Fr. Mull — *Ocnerodrilus occidentalis* Eisen — *Lampito mauritii* Kinberg — *Octochaetoides beatrix* Beddard.

Table 2. Composition of wormcasts of *L. mauritii*.

Constituent	No. of observations	$\mu g\ g\ dry\ wt^{-1}$ ($\bar{X} \pm$ S.D)	
		Control soil	Castings
Ammonia nitrogen	10	163 ± 13	268 ± 14
Urea nitrogen	8	143 ± 11	896 ± 18
Total nitrogen	4	1180 ± 66	2649 ± 86
Organic carbon	6	6209 ± 136	2181 ± 178
Oxidizable organic matter	6	692 ± 14	928 ± 29
K^+	5	163 ± 16	216 ± 28
P_i	5	179 ± 12	252 ± 18
Phenols	6	4100 ± 10	2400 ± 30
Cytokinins	5	$(0{\cdot}04 \pm 0{\cdot}01) \times 10^{-3}$	$(3{\cdot}43 \pm 0{\cdot}12) \times 10^{-3}$
Auxins	5	$(1{\cdot}11 \pm 0{\cdot}02) \times 10^{-3}$	$(43{\cdot}27 \pm 6) \times 10^{-3}$

Table 3. U/A ratios and phenol levels in the casts and the rate of plant growth promotor output by some tropical earthworms.

Species	Percent decrease in phenols by worm activations	U/A ratio of castings $(n = 8)$	Rate of production[a] (nano grams day^{-1} worm^{-1})	
			Cytokinins[b]	Auxins[c]
Wormless control soil	0·0	0·76 ± 0·05	Nil	Nil
L. mauritii	41·5	3·34 ± 0·16	4·3 ± 0·016 (5)	54 ± 6 (5)
P. elongata	26·2	2·96 ± 0·24	82·3 ± 7·16 (6)	419 ± 16 (6)
P. corethrurus	30·1	1·16 ± 0·09	67·6 ± 2·12 (5)	302 ± 14 (5)
P. excavatus	55·9	0·82 ± 0·13	78·1 ± 0·52 (6)	316 ± 21 (6)
O. occidentalis	23·8	1·34 ± 0·11	5·1 ± 0·76 (4)	76 ± 8 (4)
D. barwelli	31·7	0·96 ± 0·08	3·2 ± 0·12 (6)	48 ± 5 (6)
O. beatrix	17·1	0·86 ± 0·07	3·9 ± 0·11 (6)	42 ± 7 (6)

[a] No. of experimental observations are given in parenthesis.
[b] Benzyladenine equivalents.
[c] Indol acetic acid equivalents.

The growth promotor levels in soils of different biotopes showed a direct positive correlation with the population density of earthworms inhabiting those soils (table 4, figure 1). Greater levels were found in biotopes with higher densities of populations. These results substantiate the fact that more the worm activations, the greater will be the levels of growth promotor substances in the inhabiting soils.

The plant growth promotor activities of the wormcasts were found to be fairly stable (maintaining about 90% initial activity) for about 3 weeks under constant moisture and darkness (table 5). Aging of the casts despite these conditions destroyed the activity, and it was found that nearly 60–70% of the activity was lost in the casts aged for 10 weeks (table 5). Exposures to sunlight for 72 hr destroyed nearly 99% of the activities of cytokinins and auxins in the casts (table 6). Exposures for 15 hr generally retained 90% of the activity. Exposure timings showed inverse correlations ($\log Y$ (moisture) $\doteq 0{\cdot}2034–0{\cdot}0189\, x$ (exposure), $r = -0{\cdot}9922$) with the moisture level in the casts. Moisture level of the casts showed inverse correlations with percent loss of cytokinin activity (y(cytokinin) $= 4{\cdot}1604 - 4{\cdot}5675x$ (moisture), $r = -0{\cdot}8857$), as well as with that of auxin activity (y(auxin) $= 5{\cdot}3606 - 4{\cdot}442x$ (moisture) $r = -0{\cdot}8935$.

The influence of wormcasts on the growth promotion of the wheat seedlings was examined and the data were presented in table 7. There was some shoot elongation and shoot wet weight biomass increase in the seedlings in acid washed sand medium. This growth promotion increased slightly in a medium of mixture of garden soil and sand (1:2 proportion). When the garden soil, sand and the wormcasts were mixed in 1:1:1

Table 4. Levels of plant growth promotors in the soils of earthworm inhabited biotopes.

Biotope	Worm Density No. per M^3	Nano gram per kg dw soil: Cytokinin[a]	Nano gram per kg dw soil: Auxin[b]
Farm garden	52	1·96	14·48
Watered farm garden	86	4·86	68·91
Irrigated garden	162	5·26	84·34
Mango orchard	202	9·32	126·43
Woodland	273	11·36	206·68
Grassland	322	19·46	221·49
Desiduous forest floor	293	18·41	242·31
Ragi field (dryland)	65	2·36	42·86
Sugarcane field	78	3·63	52·56
Tank edge	121	4·26	63·18

[a] Benzyl adenine equivalents.

[b] Indol acetic acid equivalents.

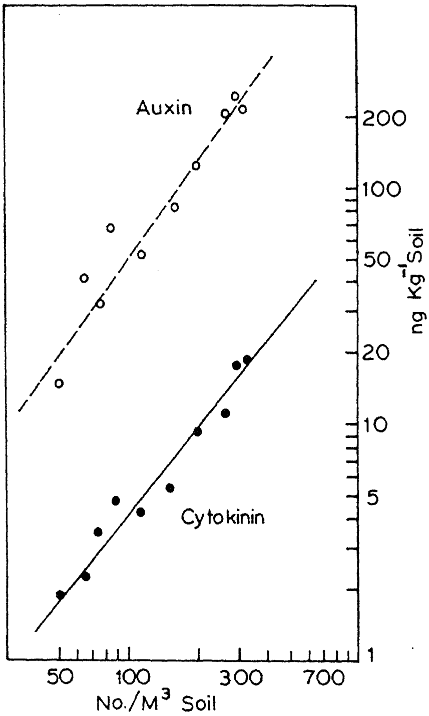

Figure 1. The relationship between the worm density of the biotope and the levels of plant growth promotors of the soil. The direct positive correlations obtained have yielded significant coefficients i.e. ($\log y = 1{\cdot}2512 \log x - 0{\cdot}7506$) $r = 0{\cdot}9669$ for auxins, and ($\log y = 1{\cdot}1615 \log x - 1{\cdot}6974$) $r = 0{\cdot}9688$ for cytokinins.

Table 5. Loss of plant growth promotor activities in the paper pulp wormcasts of *L. mauritii* on aging under constant moisture and darkness conditions at (26 ± 2°C) room temperature.

Weeks after casting	Loss of activity with reference to initial level (%)	
	Cytokinins	Auxins
0	0	0
1	2	1·5
2	4	4
3	9	7
4	15	18
5	26	21
6	35	34
7	47	41
10	69	58

Table 6. The effect of sunlight on the moisture level and plant growth promotor activities in the wormcasts of *L. mauritii.*

Exposure to sunlight (hr)	Moisture levels in the casts (%)	Loss of activity with reference to initial level (%)	
		Cytokinins	Auxins
0	24·5	0	0
10	19·4	4·4	8·6
15	11·2	6·1	9·5
24	9·2	30·7	32·3
30	5·3	51·4	54·8
48	3·3	92·5	88·9
52	2·5	98·1	97·6
72	1·1	99·5	98·9

proportion, the shoot elongation increased by 52 % and the wet weight biomass by 62 %. When sand and wormcasts were mixed in 1 : 2 proportion, the growth promotion increased further by about 72–76 %. In pure wormcast medium, the growth promotion increased still further by about 82–89 %. The wormcasts had a higher NPK ratio than the garden soil-amended medium. It could be seen from the data of table 7, that an increase in the proportion of the wormcasts despite retaining the NPK ratio constant, increased the growth promotion of the seedlings. It could be envisaged, therefore that a quantitative increase of wormcasts increased the plant growth promotor activities though not the NPK ratio.

Table 7. The effect of casts of *L. mauritii* on the growth of wheat (*T. aestivum*) seedlings during the first 15 days after sowing.

Dry weight proportion of the soil amendments				Growth promotion: Increase over the control (%)	
Acid washed sand	Wormless garden soil[a]	Worm activated garden soil casts[b]	NPK of the amendment	Shoot elongation	Shoot wet weight biomass
3[d]	0	0	0:0:0	0 (6)	0 (6)
1	2	0	0·77:0·12:0·11	0·5 ± 0·3[c] (4)	0·6 ± 0·2 (6)
1	1	1	0·83:0·08:0·09	52 ± 13 (12)	62 ± 16 (9)
1	0	2	0·85:0·07:0·08	76 ± 5 (9)	72 ± 11 (7)
0	0	3	0·85:0·07:0·08	82 ± 3 (7)	89 ± 9 (6)

[a] NPK, 0·77:0·12:0·11.
[b] Cast composition as in table 2; NPK 0·85:0·07:0·08.
[c] Mean ± S.D. of observations given in parentheses.
[d] Control amendment.

4. Discussion

Wormcasts are generally characterized by higher Eh (due to aerobic metabolism), lower pH (due to CO_2 and oxalic acids), cation exchange capacity (due to production of humic and fulvic acid), lower phenol and nitrogen contents (due to metabolism of microflora of the gut), and higher concentration of nucleic acids and phosphorus (due to the growth of microbial mass) (Hartenstein and Hartenstein 1981). It is now irrevocably accepted by the worm biologists that casting involves a pass-through of most of the soil organic matter ingested with some increase in mineralisation but probably no less than that which would occur in wormless soil *in situ*. Numerous indices have been evolved in the past such as C/N ratios, humic/fulvic acid (H/F) ratios etc to measure the extent of mineralisation that occurred due to worm activity (Kale and Krishnamoorthy 1981). Hartenstein and Hartenstein (1981) have used the content of phenols as suitable measure of humification. As humification proceeds the phenol content of the soil generally decreases. The decreases in the concentration of phenols in the present study may be explained due to the likely metabolism of microflora of the gut which are capable of decomposing simple phenolic compounds (Neuhauser *et al* 1978). Species differences with references to decrements of phenolic content as envisaged in the present study will characterize their gut microflora. Some phenols present in the soil medium will also condense into humic and fulvic acids during production and aging of the wormcasts. This possibility however cannot be ruled out. Many classes of phenols inhibit the functions of plant growth hormones (Sembdner *et al* 1980) and in the light of this fact the reduction in phenol levels in the casts, has a biological value in promoting the plant growth.

Whether the earthworm secretes digestive enzymes is still an enigma. But the accumulating evidence suggest that besides its own digestive enzymatic set up, the microflora in its gut secrete most of the enzymes that play a major part of digestion (Satchell and Martin 1984; Satchell *et al* 1984). Therefore, the castings consist not only of the defaecated products of worms, but also of the intermediary metabolites of the microflora in the worms gut.

Parle (1963) observed a steady decline in the ammonia nitrogen content of the casts as they age, and consequently the nitrogen content of them increases steadily. It is conceivable that the ammonia is only an intermediary product of nitrogen metabolism of the microflora harbouring the worm's gut. The species specific variations found in the present study with reference to U/A ratios of the casts possibly would reflect the nature of the nitrogen metabolism of the microflora existing in the gut of the worm species. However, the origin of urea and ammonia in the casts is not of much importance in so far as one is concerned with the end product which has an applied objective i.e. the production of stabilized residues as a plant growth medium.

Near the soil surface the castings increase the chances of germination of seeds that are already burried in soil (Grant 1983). The present results offer evidence that plant growth promotors help the rapid growth of the plants despite the adversaries of high ammonia content of the castings.

Both cytokinins and auxins are photolabile and relatively unstable *in vivo* as well as *in vitro* systems (Sembdner *et al* 1980). In an endogenous system of plant they are destructible metabolically by the plant cell enzymes but *in vitro* they are destroyed by the specific enzymes of the soil microbes. Many microorganisms secrete them (Sembdner *et al* 1980). Not withstanding these properties, the present results have demonstrated that the wormcasts maintain the activity of these promotors for a considerable period under proper moisture level and darkness. The maintenance of these levels in the wormcasts seem to be age-dependent and moisture-specific.

The results also indicated that wormcasts have all the nutrients that are required for a plant but claims on their relative proportion to qualify the casts as a field fertilizer are still immature. The data of table 7, particularly demonstrate that the wormcasts have a greater biological value of promoting the growth of wheat seedlings than having a NPK fertilizer value. The total nitrogen content of the casts increased more than that of the surrounding medium in the present study. Changes in mineral contents observed in the study due to worm activation corroborates the earlier findings (Lee 1983). However, it should be envisaged that the cast composition varies with soils and with the levels of their humification (Lee 1983). When the entire culture medium was examined the wormcast composition differed with that of wormless medium. When individual castings were collected and compared, the situation depicted a different picture. The fresh leaf-litter amendment in the present study had a NPK ratio of 0·77:0·12:0·11. Due to worm activation in the form of castings, this ratio was changed to 0·72 : 0·15 : 0·13. In Canada, Tomlin (1983) noticed a NPK ratio of 0·61 : 0·08 : 0·16 in the wormcasts. The field fertilizers have generally a higher NPK formulation. Compared to them the castings of *L. mauritii* have a limited fertilizer value. However, it has been shown that plants grown in such vermicompost show a lush growth (Keshavamurthy 1978). The lush growth may be due to the occurrence of plant growth promotors besides due to the readily available or assimalable nitrogen present in the casts (present results). These growth promotors may be of microbial origin but the rate of their output through casts differs from species to species. The occurrence of growth promotors is further emphasized by the direct relationship noticed between the promotor levels and worm densities in soils.

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The physico-chemical environment and the plankton of managed ponds in Haryana, India

R N SINGHAL, SWARN JEET* and R W DAVIES

Aquatic Ecology Group, Department of Biology, The University of Calgary, Calgary, Alberta T2N 1N4, Canada

*Department of Zoology, Kurukshetra University, Kurukshetra 132 119, India

MS received 26 December 1984

Abstract. An investigation was made of the physical, chemical conditions of the water and substrate of nursery-cum-research-cum-rearing-cum-stocking ponds at Central Inland Fisheries Research Institute, Karnal. Temperature, pH, CO_2, dissolved oxygen, chloride, phosphate, alkalinity, nitrates, organic matter, total solids, turbidity, electric conductivity, nitrogen, P_2O_5, sand, silt, clay, $CaCO_3$, etc. were measured monthly. Statistical analysis of these factors showed some significant correlations. The phyto- and zooplankton were also sampled and the seasonal changes in abundance recorded.

Keywords. Plankton; physico-chemical properties; substrate; pond; phyto-plankton; zooplankton.

1. Introduction

Early attempts to explain lentic plankton communities attempted to relate the biota to a variety of water quality parameters e.g. Noland (1925) and Ward (1940). The distribution of certain species were correlated with pH (Carter 1971; Sprules 1975; Sharma 1981), salinity (Moore 1952; LaBarbera and Kilham 1974; Singhal and DattaGupta 1980; Yoshioka 1982), temperature (Vijayraghavan 1969; Vasisht and Sharma 1975), or dissolved oxygen (Hutchinson 1975). However, most species have rather broad tolerance ranges and, correlations with physico-chemical parameters have little predictive value except at extreme ranges (Macan 1961; Yongue *et al* 1973; Moore 1978; Bamforth 1980).

In India, pond ecology has been studied by Ganapati (1943), Alikunhi *et al* (1955), Moitra and Mukherji (1972), Badola and Singh (1981), Dobriyal and Singh (1981) and Singh *et al* (1982), but these studies were restricted to one season of the year. More detailed studies were carried out by Vasisht and Sharma (1975) on a typical urban pond in Ambala (Haryana), Mathew (1975) on Govindgarh lake, Rewa, Madhya Pradesh, Khan and Siddiqui (1977) on perennial fish ponds in Aligarh (UP), and Sharma (1980) in Kumaun lakes. There is thus very little information available on the ecology of plankton in managed ponds of Haryana and this study is an attempt to partially rectify this deficiency.

2. Material and methods

2.1 *Study area*

The study was conducted during 1982 and 1983 at Karnal. Karnal is situated on the southern bank of the Yamuna Canal at 29°45′N lat. and 77°0′E long. in the Karnal district of Haryana State, approximately 252 m above sea level.

The climate of Karnal is more or less tropical with only 3 seasons—winter, summer and rainy. The winter season starts in November, whereas, the summer and rainy seasons begin in March and July, respectively. The minimum and maximum temperatures, and average annual rainfall in the area are 3·2°C, 41·2°C and 210 mm.

The total area of the farm of the Central Inland Fisheries Research Institute, Karnal is 1·3 ha with a water surface of 1·09 ha. There are 5 stocking ponds each with an area of 0·1 to 0·23 ha and mean depth of 1·0 m. There are also 7 nursery ponds and two rearing ponds. Each nursery pond measures 0·02 ha with an average depth of 0·06 m and each rearing pond has an area of 0·07 ha with an average depth of 0·8 m. The stocking ponds at Karnal have a perennial source of water supply through a feeder channel from the main western Yamuna canal. The nursery and rearing ponds are seasonal and receive water supply through an inlet from a tubewell. There are no drainage channels. Water is pumped out of the ponds during the early winter months to permit clearing.

The bottom of the ponds is covered with alluvium. The aquatic macrophytic flora in the study ponds is not rich and is restricted to a depth of 0·2 m. The benthos consists of Oligocheta, Mollusca, Insecta and Nematehelminthes.

2.2 *Analysis of physico-chemical properties*

The conductivity, water temperature, pH, free carbon dioxide, dissolved oxygen, chlorides, alkalinity, total solids, turbidity, nitrates, phosphates and organic matter were recorded weekly using standard methods (APHA 1971). Characteristics of the substrate such as pH, organic contents, available N_2, available P_2O_5, total N_2, total P_2O_5, sand, silt, clay and $CaCO_3$ were recorded every 3 months from four 15–20 cm deep samples taken from the bottom of the ponds with an Ekman dredge. These analyses were also carried out using standard methods (APHA 1971). Monthly plankton samples were collected with Freshwater Biological Association standard conical bolting silk plankton-nets (70 meshes/cm) with a 30·5 cm diameter mouth from 14 (5 stocking, 7 nursery and 2 rearing) ponds. As no significant differences were found between the ponds, data from only one is presented as representative of all the ponds. Vertical hauls were taken and the plankters identified to genus, counted using a Sidgewick Rafter Counting cell and categorized as abundant, common, rare or accidental. Plankters which made up more than 50% of the total were classified as abundant; those making up less than 50% but more than 15% were classified as common; those making up less than 15% as rare; and those recorded only occasionally were classified as accidental.

3. Results

3.1 *Temperature*

Water temperature varied from 12·5° (January) to 38·5°C (July through September) (table 1), with the rapid decrease in water temperature in November indicating the start of winter. The average difference between air and water temperatures was 5°C.

3.2 *pH, free carbon dioxide and dissolved oxygen*

The pH of the water in all the ponds ranged from 7·3 in February to 8·2 in May and readings taken 1 cm above the substrate surface showed no differences from those

Table 1. Monthly mean readings of the physico-chemical parameters measured in a pond at Central Inland Fisheries Research Institute, Karnal.

Months	Water Temperature (°C)	pH	Free CO_2 (ppm)	Dissolved oxygen (ppm)	Chlorides (ppm)	Alkalinity (ppm)	Total solids (ppm)	Turbidity (ppm)	Nitrates (ppm)	Phosphates (ppm)	Organic matter (ppm)
August, 1982	31·0	8·0	6·10	6·00	9·2	129·0	140	48·0	0·16	0·48	0·12
September	31·5	7·8	6·70	8·75	8·6	134·0	136	65·2	0·17	0·49	0·16
October	28·5	8·0	7·04	8·25	10·3	138·0	800	67·5	0·21	0·56	0·17
November	20·5	7·7	7·03	13·25	14·2	138·0	902	63·2	0·19	0·52	0·13
December	15·5	7·4	6·80	8·95	16·0	139·0	1003	58·2	0·18	0·41	0·14
January, 1983	12·5	7·4	5·00	9·98	20·0	139·2	1088	35·5	0·13	0·38	0·12
February	16·5	7·8	3·00	10·59	22·0	139·4	1106	34·8	0·11	0·45	0·15
March	30·0	8·2	0·00	18·25	33·00	141·0	1498	20·1	0·05	0·42	0·07
April	30·0	8·0	2·10	8·15	38·0	143·0	1552	15·0	0·00	0·20	0·00
May	30·0	8·2	3·30	11·08	33·0	144·2	1430	30·2	0·08	0·35	0·09
June	27·8	8·0	5·40	5·90	32·0	148·0	1398	30·1	0·11	0·38	0·01
July	38·5	8·0	5·90	5·44	31·0	109·0	1200	60·2	0·15	0·45	0·12

taken in the surface water. Free carbon dioxide varied between 0·0 (March) and 7·04 ppm (October). The dissolved oxygen concentration ranged from 3·15 (March) to 18·25 ppm (April) (table 1).

3.3 *Chlorides, phosphates and alkalinity*

The chloride and phosphate concentrations ranged from 8·6–38·0 ppm and 0·30–0·55 ppm, respectively. The maximum chloride concentration was recorded in April and minimum in September. Alkalinity showed no seasonal trends and varied between 108 (July) and 146 ppm (June).

3.4 *Nitrates and organic matter*

Nitrate concentration in the ponds varies between 0 and 0·21 ppm and the organic matter varied between 0·0 (April) and 0·17 ppm (October). Particulate organic matter concentrations were sometimes less than the concentration of dissolved organic matter.

3.5 *Total solids and turbidity*

Total solids showed little variations ranging from 134 (September) to 1552 ppm (April). Turbidity ranged from 15·0 (April) to 67·5 ppm (October).

3.6 *Chemical composition of the substrate*

The organic content of the pond substrate is largely colloidal (humus) and varied between 0·031% (March) and 1·282% (October) (table 2). The pH varied from 7·0 (August and March) to 7·2 (January). The electric conductivity range was from 0·23–0·44 mmh/cm.

Available nitrogen and P_2O_5 varied from 21·38–38·98 mg/100 g and 2·36–10·35 mg/100 g, respectively. Available N_2 was recorded maximum in October and minimum in April. However, total N_2 was reported to be maximum (0·760 mg/100 g) in December and minimum (0·084 m/100 g) in April, whereas available P_2O_5 was found to be maximum in October and minimum in March (table 2).

The percentage sand, silt and clay of the dried soil of the ponds showed little variation, ranging from 32–44%, 25–30% and 26–38%, respectively. The percent values of $CaCO_3$ in the substrate varied between 5·5 in July and 14·5 in December.

3.7 *Correlation and regression*

Data on the measured physico-chemical properties of the water and substrate were analyzed to determine whether correlations occurred between parameters. The correlation matrix of physico-chemical properties of water indicated that water temperature is significantly positively correlated to atmospheric temperature

Table 2. Monthly mean readings of the physico-chemical properties of the substrates measured in a pond at Central Inland Fisheries Research Institute, Karnal.

Months	Electric conductivity mmh/cm	pH	Organic content (%)	Available N_2 (mg/100g)	Available P_2O_5 (mg/100g)	Total N_2 (mg/100g)	Sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)
August	0·44	7·0	1·121	26·98	4·21	0·098	36	27	29	8·5
September	0·43	7·1	1·213	31·28	9·90	0·154	36	28	31	9·1
October	0·23	7·0	1·282	38·98	10·35	0·531	35	29	30	8·9
November	0·35	7·1	1·101	33·10	10·12	0·632	33	29	34	10·8
December	0·29	7·1	0·980	34·30	9·82	0·760	32	30	38	14·5
January, 1983	0·41	7·2	0·781	30·10	10·22	0·562	32	30	38	14·5
February	0·28	7·1	0·098	25·12	7·98	0·403	39	30	38	14·5
March	0·36	7·0	0·031	24·24	2·36	0·092	42	30	38	12·5
April	0·30	7·0	0·987	21·38	4·21	0·084	44	26	34	5·5
May	0·28	7·1	1·212	26·21	6·31	0·097	44	25	26	5·5
June	0·39	7·2	1·501	30·10	7·21	0·154	43	25	26	5·5
July	0·42	7·0	0·923	28·40	8·28	0·098	42	26	26	5·5

($r = 0.941$; $P > 0.01$), and is negatively correlated with dissolved oxygen concentrations ($r = 0.713$; $P > 0.05$). Excepting for the positive correlation with water temperature, pH exhibited no other significant (positive or negative) correlations. Free CO_2 is negatively correlated to chlorides and total solids and positively correlated to turbidity, nitrates, phosphates and organic matter. Chlorides are significantly negatively correlated with turbidity, nitrates, phosphates and organic matter; and positively correlated to total solids. Total solids in the ponds are positively correlated with turbidity, nitrates, phosphates and organic matter.

The correlation matrix for the substrate parameters shows several significant correlations. Electric conductivity is negatively correlated with pH ($r = -0.589$) and the organic content is positively correlated with available N_2 and negatively correlated with silt, clay and $CaCO_3$.

There is a positive significant correlation between available N_2, available P_2O_5, and total N_2 but is negatively correlated with sand. The available P_2O_5 is negatively correlated with total N_2 and sand while total N_2 has a positive correlation with silt and $CaCO_3$, and a negative correlation with sand.

The data on the physico-chemical properties of the water and the substrate were also statistically analyzed for simple regressions.

The mean values of the physico-chemical properties of the water and the substrate were also used in multiple regression analysis (tables 3 and 4).

3.8 *Biological features*

Table 5 contains the names of the various phytoplankton and zooplankton recorded in the ponds. *Chlamydomonas*, *Chlorogonium* (Protozoa: Mastigophora) and *Peridinium* are abundant in the ponds almost throughout the year whereas many species of Algae, Protozoa, Rotifera and Copepoda (table 5) are commonly found in the warmer months of the year. On the basis of the number of different plankters occurring in the ponds at different parts of the year they are categorized as abundant, common, rare and accidental (table 5). The fishes which are stocked and cultured in the ponds include *Catla catla*, *Labeo rohita*, *L. calbasu*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Cyprinus carpio*. In addition to these fishes, the other fishes which are cultured and found in the study ponds in different months are *Ophiocephalus*, *Mystus sor*, *M. scanghala*, *M. tengara*, *Barbus* and *Wallago attu*.

4. Discussion

The physico-chemical properties of water are important determinants of an aquatic ecosystem although they are greatly influenced and modified by climate and riparian vegetation (Hutchinson 1975). The nursery-cum-research-cum-stocking ponds of Central Inland Fisheries Research Institute, Karnal exhibit some similarity in terms of some of their physical and chemical properties of the water and substrate with other water bodies in Haryana but differ appreciably in terms of others. Sharma (1981) reached a similar conclusion in his earlier work on the two natural tanks of Kurukshetra and Jyotisar.

The fluctuations in water temperature in the ponds at Karnal are almost identical

Table 3. Significant ($p > 0{\cdot}01$) multiple regressions for the physico-chemical parameters of the water from a pond at Central Inland Fisheries Research Institute, Karnal.

Dependent variables	Values of A	Independent variables (X)	Values of R
Water temperature	144·460	X_1 – pH	0·916
		X_2 – Turbidity	
		X_3 – Dissolved oxygen	
		X_4 – Alkalinity	
		X_5 – Carbon dioxide	
pH	6·811	X_1 – Air temperature	0·816
		X_2 – Water temperature	
		X_3 – Chlorides	
		X_4 – Total solids	
Turbidity	−15·333	X_1 – Water	0·975
		X_2 – Free carbon dioxide	
		X_3 – Nitrates	
		X_4 – Phosphates	
		X_5 – Organic matter	
Phosphate	0·309	X_1 – Carbon dioxide	0·921
		X_2 – Turbidity	
		X_3 – Nitrate	
		X_4 – Organic matter	
Free carbon	4·076	X_1 – Turbidity	0·974
		X_2 – Nitrates	
		X_3 – Phosphates	
		X_4 – Organic matter	
Total solids	−697·317	X_1 – pH	0·925
		X_2 – Dissolved oxygen	
		X_3 – Chlorides	
		X_4 – Alkalinity	
Organic matter	0·336	X_1 – Carbon dioxide	0·923
		X_2 – Dissolved oxygen	
		X_3 – Turbidity	
		X_4 – Nitrates	
		X_5 – Phosphates	

with those reported by Sharma (1981) in the natural tanks at Jyotisar and a similar range of temperature (11 ± 1–32 ± 1°C) was recorded by Das and Srivastava (1956), George (1966) and Vasisht and Sharma (1975). Alikunhi *et al* (1955) reported a small variation of water temperature from 29°C to 37·2°C in the ponds at Cuttack, Orissa; Chacko and Krishnamurthy (1954) found the range from 34·2–42·8°C in their ponds in Madras and Vijayraghavan (1969) reported the range of water temperature from a minimum of 25°C to a maximum of 34°C in a natural pond at Madurai.

The major physico-chemical features at Karnal were the alternation between the dry season high concentrations and the relatively lower wet season concentrations of major chemicals. This fluctuation is caused by the annual rainfall being restricted to a few contiguous months, coupled with a high evaporation rate. The pond water was alkaline throughout the period of study and the pH also had a narrow range. An almost similar range of variation in pH (7·4 ± 1–9·0 ± 1) has been recorded by Das and Srivastava (1956), Moitra and Bhowmick (1968), Nayar (1970) and Sharma (1981). It was generally

Table 4. Significant ($p > 0{\cdot}01$) multiple regressions for the physico-chemical parameters of the substrate from a pond at Central Inland Fisheries Research Institute, Karnal.

Dependent variables (Y)	Values of A	Independent variables (X)	Values of R
Available nitrogen	−1·272	X_1 – Organic content X_2 – Available P_2O_5 X_3 – Total nitrogen X_4 – Silt X_5 – $CaCO_3$	0·877
Available P_2O_5	670·860	X_1 – pH X_2 – Organic content X_3 – Available nitrogen X_4 – Total nitrogen X_5 – Silt	0·866
Total nitrogen	2·306	X_1 – pH X_2 – Available P_2O_5 X_3 – Silt X_4 – Clay X_5 – $CaCO_3$	0·889
Clay	68·839	X_1 – pH X_2 – Total nitrogen X_3 – Silt X_4 – $CaCO_3$	0·822
Silt	16·226	X_1 – Available nitrogen X_2 – Available P_2O_5 X_3 – Total nitrogen X_4 – Clay X_5 – $CaCO_3$	0·955
Calcium carbonate	−114·263	X_1 – pH X_2 – Total nitrogen X_3 – Silt X_4 – Clay	0·975

seen that the higher pH values in the ponds at Karnal coincided with the periods of greater photosynthetic activity which received support from the relationship between the estimation of dissolved oxygen content and pH. Vasisht and Sharma (1975) also reported that a high pH value always coincided with phytoplankton abundance peaks in the ponds they studied.

The dissolved oxygen content showed numerous fluctuations throughout the period of study in each pond. The highest peaks of dissolved oxygen in each pond at Karnal were recorded during winter, when colder water has a greater capacity for holding dissolved gases (Hutchinson 1975). The depletion of oxygen content which occurred in August could be attributed to low photosynthetic activity or respiratory activity of heterotrophic organisms overbalancing the photosynthetic production of oxygen. The concentration of free CO_2 was not dependent upon temperature but was low when the aquatic vegetation was fully developed and was high when the ponds were flooded with rain water. In contrast, Singh *et al* (1982) reported that the concentration of free CO_2 was low when the water temperature of river Nayar was also low. The increase in the values of specific conductivity during summer could be attributed to rise in temperature and decrease in water level due to evaporation. The significant correlation between

Table 5. Seasonal abundance of the zoo- and phyto-plankton collected from a pond at Central Inland Fisheries Research Institute, Karnal.

Planktons	Abundant	Common	Rare	Accidental
Cynophyceae				
Agmenellurin	—	—	—	×
Holopedia	—	—	×	—
Gomphosphaeria	—	×	—	—
Anacystis	—	—	—	×
Spirulina	—	×	—	—
Coccochloris	—	—	×	—
Anabaena	—	—	×	—
Oscillatoria	—	—	×	—
Bacillariaceae				
Cyclotella	—	—	—	×
Coscinodiscus	—	—	—	×
Helosira	—	—	—	×
Synedra	—	—	×	—
Diatoma	—	—	×	—
Pleurosigma	—	×	—	—
Navicula	—	×	—	—
Amphora	—	×	—	—
Chlorophyceae				
Netrium	—	×	—	—
Closterium	—	×	—	—
Closteridium	—	×	—	—
Cosmerium	—	×	—	—
Micrasterias	—	—	×	—
Spirogyra	—	—	×	—
Scenedesmus	—	—	—	×
Selenastrum	—	—	×	—
Pediastrum	—	×	—	—
Actinastrum	—	—	×	—
Tetraedron	—	—	×	—
Mastigophora				
Chlamydomonas	×	—	—	—
Trachelomonas	—	×	—	—
Euglena	—	×	—	—
Chlorogonium	×	—	—	—
Phacus	—	—	—	×
Dinoflagellates				
Peridinium	×	—	—	—
Rotifers				
Trichocerca	—	—	×	—
Synchaeta	—	—	×	—
Brachionus	—	×	—	—
Pedalion	—	×	—	—
Filinia	—	×	—	—
Asplanchna	—	×	—	—
Keratella	—	×	—	—
Cladocera				
Ceriodaphnia	—	—	×	—
Copepoda				
Cyclops	—	—	×	—
Liaptomus	—	×	—	—
Nauplius	—	×	—	—
Ostracoda	—	—	×	—

alkalinity, specific conductivity and temperature further confirms this finding. Increases in alkalinity due to evaporation of water have been recorded previously (Hazelwood and Parker 1961) but the marked decline during the rainy months in the ponds at Karnal were probably due to dilution. Similar observation of decrease in methyl orange alkalinity (bicarbonate) and electrical conductivity due to rain water or the influx of flood water has also been reported elsewhere (Sreenivasan *et al* 1964) who also reported a positive correlation between the electric conductivity and the bicarbonate alkalinity of hypolimnetic water. Moreover, the specific conductance which also indicates ion concentration has been reported to be closely related to total alkalinity (Hem 1959).

Dunn (1962) while differentiating the trophic levels of Danish lakes classified the lakes having conductivity greater than 200 μmm/cm as clearly eutrophic and those with values less than 200 μmmh/cm as oligotrophic. If Dunn's criterion is applied to the ponds at Karnal their conductivity values which range between 451 and 1560 μmmh/cm falls would categorize them as highly eutrophic. Moyle (1946) suggested 40 ppm of alkalinity as a natural separation point between soft and hard water in which case the water in the ponds at Karnal would be classified as hard.

Very few reports on the occurrence of plankton from ponds in Haryana are available for comparison. Sharma (1981) recorded the abundance of Cladocera, Copepoda and Rotifera from unmanaged ponds in Kurukshetra during the winter months of November through March. Vasisht and Sharma (1978) only recorded the abundance of the Rotifera from ponds in Ambala for two months (February, March). These authors also found Cyanophyceae, Chlorophyceae, Protozoa, Rotifera, Cladocera and Copepoda commonly present in managed ponds at Karnal throughout the year except for January but only *Chlamydomonas*, *Chlorogonium* and *Peridinium* were abundant from November through March.

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Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India)

D N SAKSENA and N KULKARNI
School of Studies in Zoology, Jiwaji University, Gwalior 474011, India

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Abstract. Five polymorphic forms, including a new one, of the brachionid rotifer—*Brachionus quadridentatus*, are described from the Morar channel in Gwalior. These are the nominate form *quadridentatus*, form *cluniorbicularis*, form *rhenanus*, form *brevispina* and form *monospina* nov. The variation in different morphological forms is due to the emergence, development and elongation of postero-lateral and postero-median spines, and changes in the size of lorica. The present communication forms the first report of polymorphosis in *B. quadridentatus* from India.

Keywords. Rotifera; *Brachionus quadridentatus*; polymorphosis; morphometry.

1. Introduction

Seasonal on cyclic variations in morphology have been described in several animals viz dinoflagellates, rotifers, cladocera, protozoa and copepods inhabiting aquatic systems. This phenomenon of form variation was referred as cyclomorphosis by Lauterborn (1904). Hutchinson (1967) reviewed the occurrence of this phenomenon in all groups of zooplanktonic animals in much greater detail. Wesenberg-Lund (1900), Kratzschmar (1908), Hermann (1920), Woltereck (1928), Pejler (1957), Gallagher (1957), Butchner *et al* (1957), Nayar (1965), Arora (1966), Fukusho and Iwamoto (1981), Saksena and Sharma (1981, 1984) and Sharma and Saksena (1984) have described form variation in certain rotifers. Recently, Pejler (1980) has reviewed the subject of form variation in the rotitorian genus *Keratella* and discussed factors responsible for the variations. In the present paper, an attempt has been made to describe polymorphic forms of a brachionid rotifer, *Brachionus quadridentatus* (Hermann) collected from Morar sewage channel in Gwalior and forms first such report on this species of rotifera from India.

2. Materials and methods

Zooplankton samples were collected from Morar sewage channel, a free flowing water body running through Morar, a sub-city of Gwalior. Besides collecting sewage from various localities throughout its course in Morar, channel receives sewage water from Ramaua reservoir. The plankton samples were collected by filtering water through Birge conical bolting nylone net (No. 20) and were fixed with 5% formaldehyde. The figures were drawn with the help of Camera-lucida and measurements were taken with ocular disc duly calibrated with stage micrometer. The morphometric data of different forms of *B. quadridentatus* are presented in tables 1 and 2.

Table 1. Morphometric data of various forms of *B. quadridentatus* (mm)

Specifications	Form I	Form II	Form III	Form IV	Form V
Total length	0·20	0·19	0·21	0·23	0·34
Length of lorica	0·17	0·12	0·135	0·15	0·16
Width of lorica	0·18	0·16	0·185	0·19	0·20
Length of antero-median spine	0·03	0·02	0·03	0·035	0·05
Length of antero-intermediate spine	0·01	0·005	0·01	0·01	0·02
Length of antero-lateral spine	0·02	0·02	0·02	0·02	0·03
Length of postero-lateral spine	—	0·005	0·02 (Right only)	0·04	0·09

Table 2. Showing relative data of lorica and spines of various forms of *B. quadridentatus*.

Specifications	Form I	Form II	Form III	Form IV	Form V
Total length/length of lorica	1·18	1·58	1·56	1·53	2·13
Length of lorica/width of lorica	0·94	0·75	0·73	0·79	0·80
Length of postero-lateral spine/ length of lorica	—	0·04	0·15	0·27	0·56
Length of antero-median spine/ Length of postero-lateral spine	—	4·00	1·50	0·88	0·56
Length of antero-median spine/ length of lorica	0·18	0·17	0·22	0·23	0·31

3. Observations

In all 5 distinct polymorphic forms of *B. quadridentatus* were recognised (figure 1). For the sake of convenience in description, these forms are referred as I, II, III, IV and V and are described as under:

3.1 *Form* I—*B. quadridentatus* f. *cluniorbicularis* Skorikov, 1894

There are 6 occipital spines of which median spines are longer and curved a little outwardly. Lorica is broader than long. This form is characterised by the absence of postero-lateral spines and rounded posterior corners (figure 1A).

3.2 *Form* II—*B. quadridentatus* f. *rhenanus* Lauterborn, 1893

In this form the lateral and median occipital spines are almost equal in length. Intermediate spines are short. Two pairs of posterior spines are present. Postero-lateral spines are in the form of small buds only. The postero-median spines are thus longer than the postero-lateral spines (figure 1B).

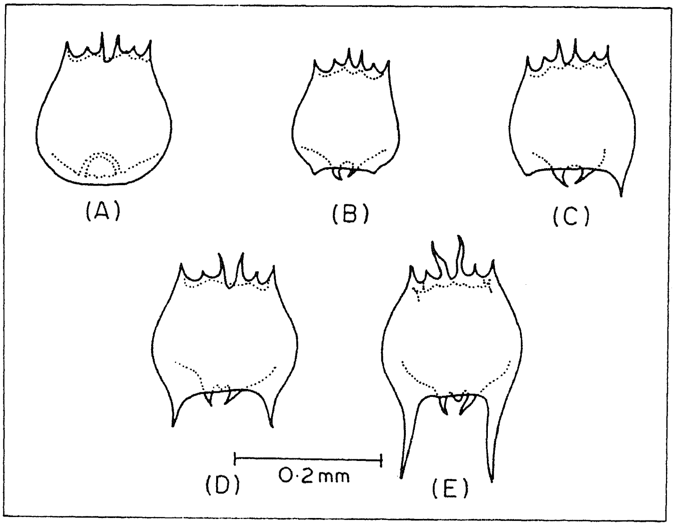

Figure 1. Different forms of *B. quadridentatus*. **A.** Form I—*cluniorbicularis*. **B.** Form II—*rhenanus*. **C.** Form III—*monospina*. **D.** Form IV—*brevispina*. **E.** Form V—*quadridentatus*.

3.3 *Form* III—*B. quadridentatus* f. *monospina* nov.

In this form due to the increase in the width of lorica the ratio between its length and width becomes less. Only right postero-lateral spine of moderate length is developed. This form is being reported for the first time (figure 1C).

3.4 *Form* IV—*B. quadridentatus* f. *brevispina* Ehrenberg, 1832

This is characterised by the presence of postero-lateral spines of moderate length on both the sides, as well as postero-median spines. Lorica is wider (figure 1D).

3.5 *Form* V—*B. quadridentatus* f. *quadridentatus* Hermann, 1783

This is the nominate form of *B. quadridentatus* having well developed anterior and posterior spines. Antero-median spines are larger than antero-lateral spines. Length of postero-lateral spines increases with the increase in the size of lorica (figure 1E).

The variations in different forms of *B. quadridentatus* are due to initial appearance and subsequent development and elongation of postero-lateral and postero-median spines. The variations are also in the antero-median spines, whose length increases gradually from forms I–V. With the increase in the width of lorica, the relative length of lorica decreases. Morphometric data of different morphological forms are given in tables 1 and 2.

Following are the prominent morphometric changes in lorica and various spines in various forms of *B. quadridentatus*:

(i) Total length of lorica increases from forms I–V (i.e., 0·20–0·34 mm).
(ii) Width of lorica also increases gradually from 0·18–0·20 mm from forms I–V.
(iii) Length of antero-lateral and antero-median spines increases gradually and reaches its maximum in form V.
(iv) The retro between length of antero-median spines and postero-lateral spines decreases from forms II–V.
(v) The length of antero-median spine/length of lorica increases from 0·18 mm in form I to 0·31 mm in form V.

4. Discussion

The phenomenon of morphological form variation is generally exhibited by those organisms which reproduce asexually or parthenogenetically for a longer period. Among rotifers, species of *Asplanchna, Keratella* and *Brachionus* have been reported to show form variation during their life histories (Hutchinson 1967; Koste 1978; Pejler 1980).

In *B. quadridentatus*, Kofoid (1908) had recorded many morphological forms from Illinois river, while other workers have described only 4 morphological forms (Luntz 1928; Ahlstrom 1940). These are f. *cluniorbicularis*, f. *rhenanus*, f. *brevispina* and f. *quadridentatus*. In our sample collected from Morar channel, Gwalior, besides 4 forms described earlier, a new form, viz., *monospina* has been added. *B. quadridentatus* f. *monospina* is characterised by the presence of only one i.e., right postero-lateral spine of moderate length. Sachse (1911) in his study, indicated that there is a gradual decrease in body length as the length of spines increases. Ahlstrom (1940), has, however, observed the opposite relationship. He found that the length of postero-lateral spines is directly proportional to the length of the specimens. Our observations regarding the length of postero-lateral spines are in confirmity with those of Ahlstrom (1940).

Regarding the factors influencing form variation in rotifers, it may be mentioned that it may occur due to seasonal indices, i.e., change in food, change in environmental factors such as climate and nutrients and as a defence mechanism against predators. Jennings (1900) observed short- and long spined-forms of *B. quadridentatus* from different water bodies and has correlated the shortness of spines in this species with the high electrolyte concentration of water. Kofoid (1908) reported different forms of *B. quadridentatus* in Illinois river, from June–September. He observed that f. *cluniorbicularis* with a rounded posterior margin was present only in summer and f. *rhenanus* was observed in August–September, while Sachse (1911) reported f. *cluniorbicularis* in the spring and f. *quadridentatus* in autumn. He concluded that the form variation in this species is not so regular with the seasons as in the case of *B. calyciflorus*. Form *rhenanus* from Berlin Dahlem was observed in spring and typical long-spined form *quadridentatus* from the same region in summer (Luntz 1928). In our study all 5 forms of *B. quadridentatus* were recorded in the month of June but only the nominate form e.g. *B. quadridentatus* during the months of July, August, suggesting that high temperature may probably trigger polymorphosis in this population. However, this needs to be confirmed.

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Effect of artificial aeration on the growth and survival of Indian major carps

M M VIJAYAN and T J VARGHESE

University of Agricultural Sciences, College of Fisheries, Mangalore 575 002, India

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Abstract. An experiment was conducted to study the effect of artificial aeration on the growth and survival of *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.). Aeration was effected by means of an air-lift system, which sucks water from the bottom and splashes the oxygenated water at the surface, thus facilitating circulation and aeration. All the 3 species of fish recorded slightly better growth in aerated cisterns when compared to the controls. However, the difference observed was marginal and statistically not significant due to the shorter duration of the experiment. Rohu and mrigal exhibited higher survival in aerated cisterns, while catla did not show much variation between the two sets. The overall computed fish production and the average rate of survival were significantly higher in the aerated cisterns when compared to the control.

Keywords. Aeration; air-lift system; *Catla catla*; *Labeo rohita*; *Cirrhinus mrigala*.

1. Introduction

Dissolved oxygen is the most critical factor in intensive fish culture and it significantly affects the production of fish. Application of huge amounts of fertilisers and fish feeds in heavily stocked fish ponds upsets the dynamics of dissolved oxygen. This becomes more pronounced in the early morning hours and on cloudy days resulting in severe depletion of dissolved oxygen of pond water. In addition to the problem of dissolved oxygen, toxic intermediates formed in the transformation of organic matter also inhibit growth of fish. These two factors which limit the production of fish in intensive culture, can be effectively controlled by the use of artificial aeration.

Many scientists have studied the effect of aeration on the growth and survival of fishes. Aeration was found to improve the water quality and increase fish production in white catfish (Loyacano 1974). Carp and tilapia yields were increased many folds by applying aeration (Marek and Sarig 1971; Rappaport and Sarig 1975). Hollerman and Boyd (1980) reported higher production and survival of channel catfish in ponds with aeration. Plemmons and Avault (1980) also obtained 6 tons of catfish/acre with continuous aeration. Singh *et al* (1980) reported aeration to favour feed utilisation and growth in common carp. There has been no work on the effect of aeration on Indian major carps. In view of this, the present study was undertaken to ascertain the effect of aeration on Indian major carps.

2. Materials and methods

The experiment was conducted for a period of 120 days at the fish farm of the College of Fisheries, Mangalore. Six uniform sized cement cisterns, each having an area of 50 m^2 (10 × 5 × 1 m), were used for the polyculture of Indian major carps. Water in a set of 3 cisterns was artificially aerated, while the second set of 3 cisterns without aeration acted as controls.

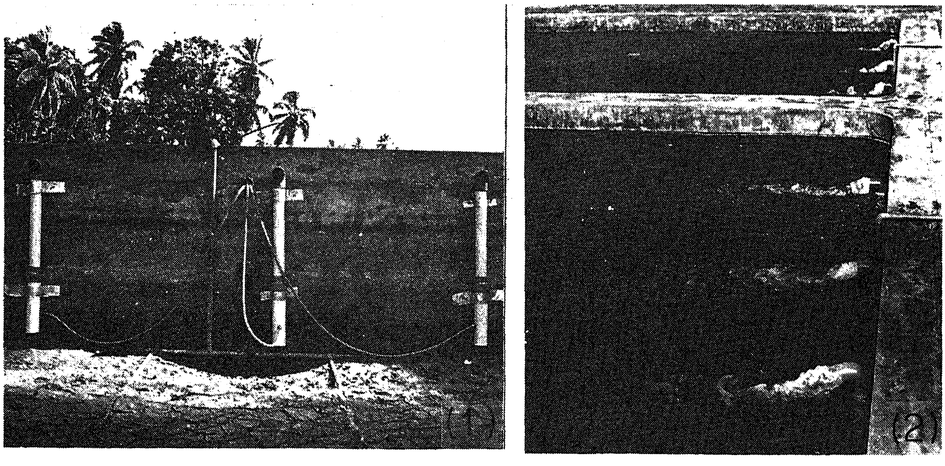
2.1 *Air-lift system*

The air-lift system used in the experiment consisted of two PVC pipes of different diameters united together and fixed vertically. The lower pipe was of a larger diameter (3″) while the upper bend was slightly smaller (2½″). Compressed air was supplied at the bottom of the vertical pipe from a 3 HP compressor through a GI pipe, which was connected to the air-lift system by a rubber tubing ending in an airstone. The flow of air through the rubber tube was regulated by means of a regulator. The air, injected at the bottom of the pipe, forms bubbles and rises resulting in an air-water mixture, which being lighter than water, gets discharged from the upper end of the pipe. The constructional and operational details of the aerating system is shown in figures 1 and 2. Three such air-lift systems were fixed in each of the 3 cisterns. Aeration was done daily for 8 hr from 23·00–07·00 hr.

2.2 *Stocking and sampling of fishes*

Prior to stocking, all the cisterns were fertilised initially with 1000 kg/ha of poultry manure on dry weight basis. Later, fortnightly application was done at the rate of 500 kg/ha. Fingerlings of catla, rohu and mrigal were stocked in each cistern at the rate of 10,000 nos/ha with a stocking ratio of 2:2:1, respectively. The fishes were fed with the conventional feed comprising of a mixture of rice bran and groundnut oil cake in equal proportion by weight at the rate of 5, 3, 2·5 and 2% in the first, second, third and fourth months, respectively.

Water and plankton samples from each cistern were analysed weekly, the results of which are being published separately. Fish sampling was done every fortnight, when a minimum of 50% of the stock was caught and individual length and total weight of



Figures 1 and 2. **1.** Full view of the air-lift system fitted in a cistern. **2.** Side view of the air-lift system showing the water being splashed.

each species recorded. On termination of the experiment, water was drained from each cistern and the surviving fishes were measured for their length and total weight. The growth and survival of Indian major carps with and without aeration were tested statistically using the analysis of variance technique (Snedecor and Cochran 1967).

3. Results and discussion

3.1 *Fish growth*

3.1.1 *Catla catla*: The average length and weight attained by catla during the experimental period in aerated and control cisterns are shown in table 1. The average length of catla in the aerated cisterns increased from 54·9 ± 0·95–229·5 ± 7·35 mm, while in controls it increased from 54·6 ± 0·70–228·2 ± 14·67 mm. The daily average increment in length was 1·46 mm in aerated and 1·45 mm in control cisterns. The average weight of catla increased from 2·0 ± 0·2–172·8 ± 19·03 g during the experiment in aerated cisterns, while in the control it increased from 2·0 ± 0·23–164·7 ± 31·81 g. The daily average increment in weight was 1·42 g in aerated and 1·36 g in control cisterns. Figure 3 shows the growth of catla in the two sets of cisterns. It can be seen that the growth of catla was marginally higher in the former. Of the 3 species cultured, catla exhibited significantly faster growth.

3.1.2 *Labeo rohita*: The data on the growth of rohu is presented in table 2. During the experimental period, the average length increased from 62·8 ± 2·16 – 221·2 ± 12·63 mm in aerated cisterns, with a daily average increment of 1·32 mm. In the control, the average length increased from 65·2 ± 2·55 – 210·7 ± 6·47 mm with a daily average increment of 1·21 mm. The average weight of rohu increased from 2·6 ± 0·15 – 131·4 ± 23·62 g in aerated cisterns and from 2·7 ± 0·17 – 103·9 ± 6·26 g in control, the average daily increment in weight being 1·07 g and 0·84 g in aerated and control cisterns, respectively. The fluctuations in the average weight of rohu over the experimental period is shown in figure 4. It can be seen from the figure that the growth of rohu was better in aerated cisterns than in controls, especially towards the later half of the experiment. The faster growth of rohu in aerated cisterns might be due to the abundance of plankton in these cisterns. Further, aeration might have favoured feed utilisation as reported by Singh *et al* (1980).

3.1.3 *Cirrhinus mrigala*: Average length and weight of mrigal obtained in aerated and control cisterns are presented in table 3. Mrigal grew from an average length of 67·2 ± 0·45–231·9 ± 10·81 mm in aerated cisterns and from 66·8 ± 1·00–226·9 ± 6·08 mm in controls. The daily average increment in length was 1·37 mm in aerated and 1·33 mm in control cisterns. The average weight increase was from 3·7 ± 0·17–125·5 ± 14·53 g and from 3·9 ± 0·46–114·1 ± 8·09 g in aerated and control cisterns, respectively. The daily average increment in weight was 1·02 g in the case of aerated cisterns and 0·92 g in control. Figure 5 depicts the growth of mrigal in terms of weight. It can be seen that the growth was faster in aerated cisterns throughout the experimental period. However, the difference became more pronounced only from the 56th day. The faster growth rate under aeration might be due to better feed utilisation. The difference in fish growth between aerated and control cisterns was statistically insignificant (table 4), probably due to the shorter duration of the experiment.

Table 1. Average growth of catla in aerated and control cisterns*.

Treatment	Growth	Days after stocking										Daily increment
		0	14	28	42	56	70	84	98	112	120	
Aerated	Length (mm) ± SD	54·9 ± 0·95	92·2 ± 3·11	117·5 ± 6·56	142·5 ± 6·21	157·1 ± 6·19	177·5 ± 4·83	193·1 ± 5·28	215·3 ± 6·29	226·8 ± 5·67	229·5 ± 7·35	1·46
	Weight (g) ± SD	2·0 ± 0·2	10·8 ± 1·50	24·2 ± 5·05	38·2 ± 4·60	51·9 ± 4·34	75·8 ± 10·37	111·2 ± 7·56	142·2 ± 12·67	165·8 ± 18·09	172·8 ± 19·03	1·42
Control	Length (mm) ± SD	54·6 ± 0·70	96·5 ± 9·52	119·9 ± 14·80	137·7 ± 16·66	155·5 ± 21·01	176·5 ± 18·22	199·7 ± 17·91	215·3 ± 11·32	224·8 ± 13·01	228·2 ± 14·67	1·45
	Weight (g) ± SD	2·0 ± 0·23	13·2 ± 5·03	25·4 ± 10·15	36·4 ± 15·44	52·0 ± 22·26	77·5 ± 28·57	120·7 ± 27·72	138·2 ± 24·22	157·3 ± 27·42	164·7 ± 31·81	1·36

*Average of 3 cisterns

Table 2. Average growth of rohu in aerated and control cisterns.*

Treatment	Growth	Days after stocking										Daily increment
		0	14	28	42	56	70	84	98	112	120	
Aerated	Length (mm) ± SD	62·8 ± 2·16	87·3 ± 4·08	115·5 ± 3·17	138·6 ± 4·90	156·0 ± 6·23	176·6 ± 6·05	193·0 ± 9·56	202·5 ± 8·50	213·9 ± 12·22	221·2 ± 12·63	1·32
	Weight (g) ± SD	2·6 ± 0·15	10·0 ± 1·74	21·0 ± 1·12	33·0 ± 4·26	48·4 ± 6·47	67·7 ± 8·07	85·3 ± 10·01	106·9 ± 18·07	124·4 ± 23·54	131·4 ± 23·62	1·07
Control	Length (mm) ± SD	65·2 ± 2·55	86·8 ± 2·05	114·8 ± 3·41	137·5 ± 7·72	153·0 ± 7·26	170·9 ± 3·99	185·3 ± 3·42	194·8 ± 3·67	202·7 ± 3·50	210·7 ± 6·47	1·21
	Weight (g) ± SD	2·7 ± 0·17	9·1 ± 1·2	20·8 ± 3·10	33·3 ± 5·42	46·8 ± 6·99	62·2 ± 5·34	79·1 ± 3·68	90·6 ± 7·32	97·8 ± 5·90	103·9 ± 6·26	0·84

*Average of 3 cisterns

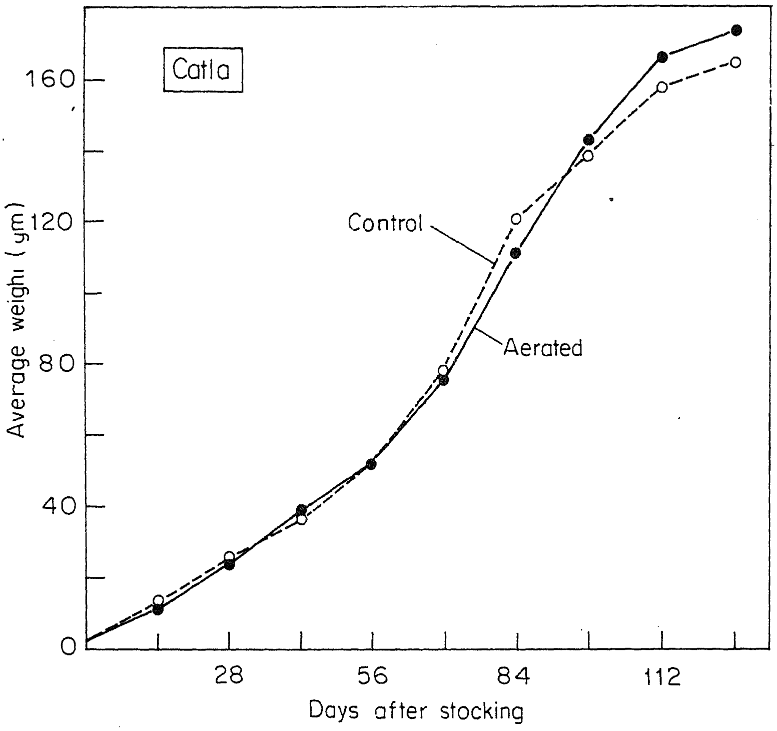


Figure 3. Average weight (g) attained by catla in the aerated and control cisterns.

3.2 *Survival and production*

The average survival of the 3 species, catla, rohu and mrigal was 84·5% in the aerated cisterns, while it was only 71·1% in the control. The survival in aerated cisterns was found to be significantly better when compared to the controls (table 4).

The average survival of catla was slightly higher in the control, where it was 93·3% while in aerated ponds it was 91·7%. Among the species, catla exhibited the best survival in both the sets; this might be because of its surface dwelling nature due to which it is not prone to severe oxygen depletion. The survival of rohu was significantly higher in aerated cisterns, where it was 75·0% while in control it was only 36·7%. This could be attributed to the higher dissolved oxygen concentration in the aerated cisterns, especially at the bottom. Since rohu is a column-bottom dwelling fish, dangerously low levels of dissolved oxygen at the bottom might have resulted in the mortality of this species in the control cisterns, especially towards the later half of the experiment. Mrigal also showed slightly higher survival in aerated cisterns (86·7%) as compared to the control (83·3%). The higher dissolved oxygen concentration in aerated cisterns might have been responsible for the better survival of mrigal in these cisterns. In comparison to rohu, the better survival of mrigal in unaerated cisterns indicates that the former species is more sensitive to oxygen depletion.

Data on the average net computed fish production are presented in table 5. The computed net average fish production in aerated cisterns was 3,710·63 kg/ha/year, while in the control it was only 2,809·82 kg/ha/year, the difference being significant.

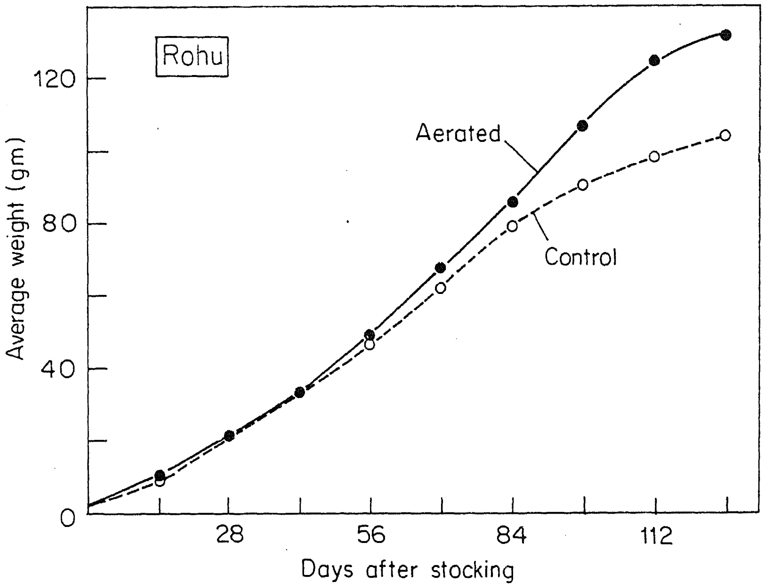


Figure 4. Average weight (g) attained by rohu in the aerated and control cisterns.

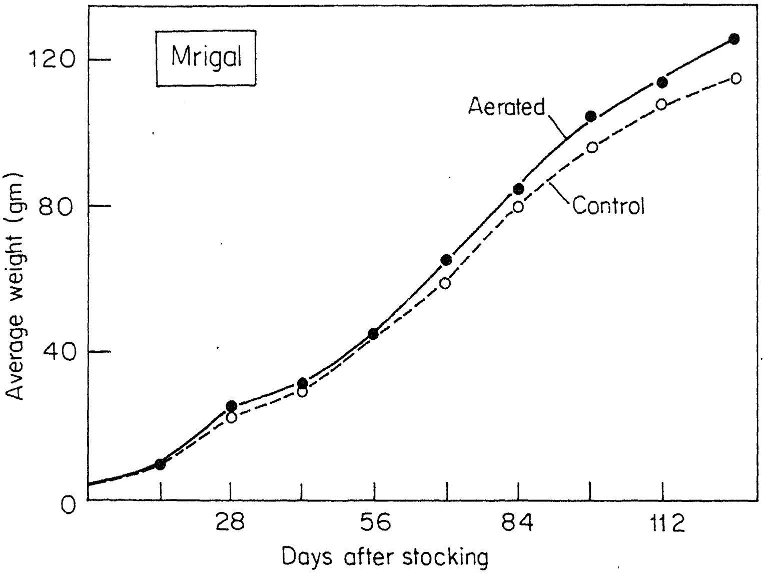


Figure 5. Average weight (g) attained by mrigal in the aerated and control cisterns.

The highest percentage contribution to the production was by catla in both the aerated (51·41%) and control cisterns (64·94%), followed by rohu (31·36%) and mrigal (17·24%) in aerated cisterns and mrigal (19·83%) and rohu (15·23%) in control cisterns.

Based on the results obtained, it can be concluded that the growth of catla, rohu and mrigal can be improved by aerating the ponds. Experiments carried out in Israel

Table 3. Average growth of mrigal in aerated and control cisterns.*

Treatment	Growth	Days after stocking										Daily increment
		0	14	28	42	56	70	84	98	112	120	
Aerated	Length (mm) ± SD	67·2 ± 0·45	88·5 ± 2·87	127·2 ± 3·22	144·8 ± 0·99	161·8 ± 5·39	183·9 ± 2·00	193·1 ± 2·72	210·3 ± 10·26	219·7 ± 5·69	231·9 ± 10·81	1·37
	Weight (g) ± SD	3·7 ± 0·17	9·7 ± 0·26	24·7 ± 2·52	31·6 ± 0·36	45·2 ± 3·01	64·9 ± 1·01	83·9 ± 1·33	104·0 ± 10·0	113·3 ± 13·32	125·5 ± 14·53	1·02
Control	Length (mm) ± SD	66·8 ± 1·00	87·5 ± 3·91	123·1 ± 3·38	140·6 ± 4·68	157·4 ± 9·05	177·5 ± 2·84	186·4 ± 3·14	203·5 ± 6·26	217·3 ± 3·33	226·9 ± 6·08	1·33
	Weight (g) ± SD	3·9 ± 0·46	9·4 ± 0·51	22·8 ± 10·58	29·7 ± 2·06	44·8 ± 6·30	58·9 ± 3·98	79·5 ± 3·12	95·3 ± 5·64	107·1 ± 5·28	114·1 ± 8·09	0·92

*Average of 3 cisterns

Table 4. Analysis of variance for survival and growth of catla, rohu and mrigal.

Source of variation	d.f.	Mean sum of squares		F-ratio	
		Survival	Growth	Survival	Growth
Between replications	2	0·39	767·27	0·19	2·59
Between treatments	1	34·72	1117·07	17·45*	3·77
Between species	2	149·56	5254·72	75·16*	17·72*
Interaction between treatment and species	2	26·89	160·93	13·51*	0·54
Error	10	1·99	296·52		
Total	17				

*Significant at 5% level.

Table 5. Net production (kg/ha/year) in different cisterns.

Treatments	Catla	Rohu	Mrigal	Total carp production
A1	1963·94	1435·42	677·81	4077·17
A2	2010·42	1232·18	653·59	3896·19
A3	1719·82	860·18	578·53	3158·53
Average	1898·06	1175·93	636·64	3710·63
C1	2054·16	369·87	493·24	2917·27
C2	1620·36	557·84	534·12	2712·32
C3	1810·40	348·70	640·76	2799·86
Average	1828·31	425·47	556·04	2809·82

showed that carp and tilapia yields could be increased several folds by aeration of pond water (Marek and Sarig 1971; Rappaport and Sarig 1975). Similarly, aeration of pond water resulted in increased production and survival of channel catfish (Hollerman and Boyd 1980; Plemmons and Avault 1980). The marginal difference observed in the growth of fish between the two sets in the present study, seems to be mainly due to the shorter duration of the experiment. It was seen that the rate of survival could be significantly enhanced by aerating fish ponds. The overall carrying capacity and fish production from ponds under intensive polyculture could be significantly increased by resorting to aeration. Further aeration experiments using all the 6 species of carps viz catla, rohu, mrigal, silvercarp, grass carp and common carp at a higher stocking density should yield more useful information on the utility of this technique for enhancing fish production from intensive aquaculture.

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Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

A MINI and V K K PRABHU

Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695 581, India

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Abstract. Final (3rd) instar larvae of the coconut rhinoceros beetle *Oryctes rhinoceros* were found to undergo precocious metamorphosis when subjected to starvation; the beetles produced as a result were subnormal in size. However, the larva showed this kind of response to starvation only after attaining a certain critical stage of development; otherwise they died after surviving for a varying period, depending on their age. Topical application of the juvenile hormone analogue 'Kinoprene' (ZR 777) in sufficient doses at definite periods caused significant delay in the onset of precocious metamorphosis thus suggesting an endocrine involvement. Evidently, starvation might have caused a decrease in JH-titre which in its turn, triggered a chain of events leading to precocious metamorphosis.

Keywords. Coconut rhinoceros beetle; *Oryctes rhinoceros;* starvation; precocious metamorphosis; critical developmental stage.

1. Introduction

Oryctes rhinoceros (Coleoptera: Scarabaeidae) is a serious pest on coconut palm in the palm-growing belt throughout the world. During our studies involving starvation of the 3rd (final) instar field-collected larvae of this animal, we noted two types of response from the larvae to starvation: (i) some of them died without entering into pupation whereas others (ii) pupated after certain number of days of starvation. As the causes involved in this differential response are significant from the point of view of control of this pest, we conducted a detailed critical study of the various factors involved in this differential response of the larvae to starvation.

2. Materials and methods

For preliminary experiment (expt I) final (3rd) instar larva with head capsule width of 10–11 mm (Nirula 1955) were collected from field and introduced on (i) cowdung, (ii) fine sand, washed repeatedly with water to remove any nutrients and (iii) mixture of cowdung and sand in 1:1 ratio by volume, 10 larvae in each group. The medium was kept moist by periodical watering. Accumulating faecal pellets were removed and medium changed for fresh one if necessary. The larvae were introduced singly in separate plastic containers (10·5 cm high and 9 cm diameter) with lid which had holes drilled on them, filled one third the volume with the medium. The timing of various developmental events viz the onset of transition into prepupa, duration of prepupal and pupal stages were noted for all the larvae by daily observations after moving the superficial layers of the rearing media, causing least disturbances to the individuals. When cocoon was formed the observations were made after carefully breaking off a part of the cocoon.

For the following experiments, 3rd instar larvae of known age groups were obtained from a stock of '0' day old 3rd instar larvae, raised in the laboratory from field collected second instar larvae (head capsule width 5 mm) and maintained on cowdung.

To find out if the age of the larva had any effect on successful metamorphosis in a sand-alone medium, larvae of different age groups (15-days, 35-days and 40-days after moulting) were introduced on sand as above (expt II).

For the expt III, 48-day old larvae obtained at a time were divided into 3 groups and each group was then introduced into any one of the following media: (i) cowdung, (ii) riversand and (iii) dung:sand mixture (1:1 ratio by volume). Various developmental events upto adult emergence as well as the weight of the adults were recorded. This experiment was replicated so that 20 larvae were studied in each medium.

During the IVth expt, development of the 48-day old larvae in two media devoid of any food substance, a medium formed of the faecal pellets collected from the rearing containers and a medium of spongy rubber bits was observed after keeping a group of larvae in a medium of cowdung for control. All the starved larvae which pupated were allowed to emerge and the adults so emerged were studied.

A 1 μg/μl solution of 'Kinoprene' (ZR 777) (propyonyl 2e, 4e–3, 7, 11–trimethyl 2, 4–dodeca dienoate) in acetone was used for topical application. The solution/acetone was applied on the thoracic region of the larvae (without anaesthesia) at different stages of starvation, obtained by introducing 48-day old 3rd instar larvae in sand. The experiments were performed in the following manner.

Expt I: 50 μg hormone analogue applied on 48 day old larvae immediately before the initiation of starvation.

Expt II: 50 μg hormone analogue applied on the 8th day of starvation.

Expt III: 50 μg hormone analogue applied on the 8th and 12th days each of starvation (i.e. total 100 μg).

Expt IV: 50 μg hormone analogue applied on the 5th, 7th, 9th, 11th and 13th days each of starvation (i.e. total 250 μg).

For each experiment, a group of larvae subjected to an identical treatment with acetone and another group of normal control (untreated) larvae were maintained. After each treatment, the larvae were returned to the respective medium. Development of all the larvae was followed and recorded.

3. Results and discussion

3.1 *Effect of various media on pupation of 3rd instar larva of unknown age*

Results of this experiment are shown in table 1. The larvae introduced on cowdung and dung-sand mixture showed a high variability in the timing of onset of metamorphosis (within 23·9 ± 16·6 and 24·7 ± 15·0 days, respectively). However, the larvae introduced on sand alone medium were found to show two kinds of response—50% of the larvae died within 21·8 ± 3·5 days without showing any signs of metamorphosis, while the others metamorphosed successfully within 12·6 ± 5·9 days. The duration of prepupal and pupal stages were similar for the 3 groups of larvae.

Table 1. The effect of different rearing media upon *O. rhinoceros* larvae of different age groups.

Expt. No.	Age of the larva (days)	Rearing medium	Mortality (%)	Time taken for initiation of metamorphosis after the beginning of experiment (days)	Duration of prepupal stage	Duration of pupal stage
		Cowdung	0	23·9 ± 16·6	9·6 ± 1·1	19·2 ± 0·9
I	Unknown	Sand	50	12·6 ± 5·9	9·2 ± 1·1	19·4 ± 1·0
		Dungsand mixture	0	24·7 ± 15·0	9·5 ± 1·4	19·3 ± 0·9
	15		100	—	—	—
II	35	Sand	100	—	—	—
	40		50	28·0 ± 2·0	11·5 ± 0·5	18·5 ± 0·5
		Cowdung	—	41·2 ± 7·3	9·8 ± 1·5	18·4 ± 0·7
		Sand	—	9·1 ± 1·5	10·4 ± 1·6	18·6 ± 1·2
III	48	Dungsand mixture	—	44·6 ± 6·4	10·0 ± 1·4	18·5 ± 0·7
		Faecal pellets	—	9·2 ± 0·8	10·0 ± 1·10	18·4 ± 0·5
IV	48	Spongy rubber	—	9·6 ± 1·0	10·0 ± 1·4	18·8 ± 0·8
		Cowdung	—	41·0 ± 4·5	9·4 ± 1·0	17·8 ± 1·3

3.2 *Effect of starvation on 3rd instar larvae of known age*

The high variability observed in the initiation of metamorphosis among the larvae introduced on the sand alone medium could be attributed to the fact that the field collected larvae were heterogeneous group of individuals of different age groups. Those that passed a critical age apparently showed a tendency to become prepupa within a few days while those which did not pass that critical age began metamorphosis only after attaining a stage of maximum growth possible for them on the medium on which they were introduced.

Some of the larvae introduced on sand alone medium died without undergoing metamorphosis while the others could metamorphose successfully, led to the assumption that there existed a certain critical stage of development for the larvae to become competent to metamorphose, those which had not reached this stage could not metamorphose and hence they died after surviving for a few days. Fifteen day old or even 35-day old larvae were found to be incompetent for metamorphosis when starved; when introduced on sand, they died after a survival for 13·3 ± 1·1 and 19·3 ± 3·6 days, respectively. Older the larvae, when they were subjected to starvation, the greater was the duration for which they survived; if the larvae were 40-day old when starvation started there was 50% mortality (table 1). Hence, it was evident that the final instar larvae may reach the critical stage of development just after the age of 40 days. However, 48-day old larvae were selected for all subsequent experiments, since they seem to be well beyond the critical stage, and all of them pupated when subjected to starvation.

3.3 *Effect of various media on pupation of 48-day old 3rd instar*

The 48-day old larvae reared on cowdung, initiated metamorphosis within 41·2 ± 7·3 days thereafter, those on river sand within 9·1 ± 1·5 days and those on dung sand mixture within 44·6 ± 6·4 days after beginning of the experiment. In other words, the larvae introduced on the sand alone medium were found to reveal a precocious onset of metamorphosis quite early. However, no significant change was observed in the duration of metamorphosis among these 3 groups of larvae (table 1). The prepupae, pupae and adult beetles produced as a result of precocious metamorphosis were much reduced in size. Whereas the controls weighed 6·2 g and those reared on dung sand mixture weighed 5·6 g, those reared on sand alone medium weighed only 3·7 g. The original larvae weighed 10·4–10·9 g.

The phenomenon of precocious pupation, induced by a sand-alone medium, could be regarded either as a response to the nature of the medium or as due to the effect of starvation induced by this medium, since it contained no organic matter that could be utilized as food. However, the larvae introduced on dung-sand mixture did not show precocious metamorphosis. Hence, it appeared that it was starvation and not the nature of the medium that induced precocious metamorphosis when the larvae were introduced in sand only. This was supported by the fact that two other media devoid of any food substance, a medium consisting of faecal pellets of the larvae collected from the rearing containers and a medium consisting of spongy rubber bits taken from worn-out foam cushions also could induce precocious metamorphosis when the larvae were introduced on them (table 1).

It may be noted that Nirula (1955) also pointed out the significance of food supply on development of *Oryctes rhinoceros* larva. However, he observed that the larval development was limited if food supply was exhausted before the end of the larval period. According to him, the two month old larvae remained alive for 14–20 days without any food, and continued larval life when food was available subsequently. He does not mention of starvation inducing pupation in the larvae. Catley (1969) also maintains that unfavourable climatic or nutritional conditions delay larval development which may be extended upto 14 months smaller than average size bettles being produced thereby. This may be correct when starvation is only partial, and when at least some nutrition is available to the animal. Present studies however clearly show the existence of a critical period in the life history of 3rd instar which is extremely important from the standpoint of survival during complete starvation. If the animal is subjected to complete starvation before the larva attains this age (40–48 days) the larva dies without pupating; after this stage, the larva will enter pupation about 9-days after starvation is induced, resulting in smaller adults ultimately. This is of paramount importance for the insect to tide over unfavourable condition imposed by starvation. It may thus be noted that the 3rd instar larvae enter pupation by about 57 days, whereas normally the control larvae take about 89 days, thereby cutting short more than 30 days of life history so that at least some of the animals can survive to reproduce, though the younger ones will succumb to adverse condition.

A precocious decline in JH-titre appeared to be the reason for the transformation of the starved larva into a precocious pupa. Application of a single dose of 50 µg of 'Kinoprene' (ZR 777) immediately before initiation of starvation did not produce any significant effect either upon the onset or duration of metamorphosis (table 2) or

Table 2. The effect of topical application of the JHA ZR 777 upon 48-day old third instar larva at different stages of starvation.

Expt. No.	Dosage and timing of application of JHA	Treatment	Time taken for the onset of metamorphosis (days)	Duration of prepupal stage (days)	Duration of pupal stage (days)
I	50 μg immediately before initiation of starvation	Hormone treated larva	10·7±0·9	9·0±0·8	18·7±1·3
		Acetone treated control larva	11·0±0·8	9·0±0·8	18·0±0·0
		Normal larva	10·3±0·5	8·7±0·9	18·7±0·9
II	50 μg on the 8th day of starvation	Hormone treated larva	12·3±1·3	8·3±0·5	18·7±1·7
		Acetone treated control larva	10·7±0·9	9·0±0·8	18·3±0·5
		Normal larva	11·0±0·8	8·7±0·5	18·7±0·9
III	50 μg each on the 8th and 12th days of starvation	Hormone treated larva	13·0±3·3	10·0±1·6	19·7±1·3
		Acetone treated control larva	9·7±2·1	9·7±0·5	18·3±0·5
		Normal larva	10·7±1·3	9·0±1·4	18·7±0·9
IV	50 μg each on the 5th, 7th, 9th, 11th and 13th days of starvation	Hormone treated larva	17·3±0·9	9·3±0·9	18·7±0·5
		Acetone treated control larva	10·7±0·9	9·7±1·3	20·0±1·6
		Normal larva	9·7±0·9	10·7±0·9	19·0±1·4

in the morphological characteristics of the prepupae, the pupae or adult beetles, when compared to the acetone-treated and normal control larvae. This ineffectiveness of the single dose of 50 μg of ZR 777 might be because exogenous hormone could not persist in the body until the endogenous JH-titre decreased due to starvation. Part of the analogue might have also been lost during movement of the larvae through the medium. When 50 μg of ZR 777 was applied to the larvae on 8th day of starvation also, no significant effect on metamorphosis was revealed (table 2). Apparently because by the time the JHA was applied, the larval pupal transition already switched on. Hence it appeared that a significant effect on metamorphosis could be expected if the JHA was applied in sufficient dose at that critical stage, after the JH has declined and before the metamorphic changes have switched on. When 50 μg was applied each on the 8th and 12th days of starvation, a single individual showed a significant delay in the onset of metamorphosis; it revealed the signs of transition into prepupa only on the 17th day of starvation while the acetone-treated and normal control larvae showed this transition within 9·7 ± 2·1 days and 10·7 ± 1·3 days, respectively. However, the remaining hormone treated larvae did not show any effect of the hormone. But when the JHA was applied repeatedly for 5 alternate days from the 5th day of starvation, 50 μg at a time, all the larvae showed significant delay in the onset of metamorphosis (table 2). Presumably, repeated application of ZR 777 at short intervals, from a fairly earlier stage of starvation, ensured the presence of this JHA at the critical stage of JH-decline in all the larvae in spite of any individual variation that might have existed in the timing of this decline. Also, repeated application might have produced a prolonged and possibly a cumulative effect. However, all the larvae began to metamorphose within a few days (3–5 days) after receiving the last dose. This indicated that the effect of 50 μg of ZR 777 could last only for a short duration.

Although the initiation of metamorphosis was delayed by the JHA, it could not affect the duration of metamorphosis; also, it did not produce any apparent abnormalities in development. These observations are in agreement with the view that in many species of Coleoptera, the usual effect of JHAs on the last larval instar is characterized by a delay of pupation; due to special hormonal condition controlling larval-pupal transformation in these insects, development in the last larval instars appears to be suspended for as long as the JH remains in the body. Such hormonal condition prevent the juvenoids from causing disturbance of metamorphosis in these species (Slama *et al* 1974).

It was thus revealed that in *O. rhinoceros*, metamorphosis could not ensue as long as the JH-titre remained high. Precocious pupation observed in the starved larvae might have hence been due to a decline in JH-titre. In otherwords, starvation appeared to induce a decline in JH-titre in these larvae.

Starvation was reported to cause a rise in concentration of JH in the blood of 5th instar *Manduca sexta* larvae (Bhaskaran and Jones 1980; Nijhout 1975) producing supernumerary larvae. However, in *Oncopeltus fasciatus* starvation caused a reduction in JH-titre in the haemolymph (Rankin and Riddiford 1977). A similar mechanism might have been in operation in the starved larvae of *O. rhinoceros*. Though starvation-induced precocious metamorphosis is extremely rare in insects, there is a striking similarity of the present findings on *O. rhinoceros* with those of D'Angalo *et al* (1941) on tadpole. They found that the stage of the tadpole at the time of food withdrawal was critical for metamorphosis. Starvation after a certain critical stage accelerated metamorphosis in tadpole, but before that stage, it caused a delay in metamorphosis.

The existence of a certain critical stage for onset of metamorphosis may be explained as due to the fact that most of the organs can achieve differentiation in response to hormones only after attaining a certain stage of development (Wigglesworth 1954). A threshold size for metamorphosis has been reported in the tobacco hornworm (Nijhout 1975) in which however, starvation of the mid 4th instar larvae produced undersized 5th instar larvae which then underwent one or more extra larval moults. Also, 5th instar larvae (2 days after the moult to 5th instar) starved at body weight of less than 4 g, underwent delayed moulting into larval-pupal intermediates (Nijhout and Williams 1974).

That starvation can evoke precocious metamorphosis may be of adaptive significance to the animal in that it enables the larvae to escape from conditions of severe food shortage by transforming itself into the adult, with an entirely different food preference.

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Prediction of absorption efficiency from food nitrogen in amphibians

T J PANDIAN and M PETER MARIAN

School of Biology, Madurai Kamaraj University, Madurai 625 021, India

MS received 26 November 1985

Abstract. Gravimetric estimation of absorption efficiency in amphibians is a time-consuming process and still subject to technical errors. The need for the application of indirect methods requiring no quantitative recovery of feces is indicated. From 41 values reported for 11 amphibian species, it is observed that nitrogen content of food is significantly ($P < 0{\cdot}001$) and positively correlated ($> 0{\cdot}9$) with absorption efficiency. From the obtained regression equation (absorption efficiency $= 49{\cdot}615 + 4{\cdot}596$ nitrogen), the per cent efficiency can be predicted with 3·748 standard error.

Keywords. Absorption efficiency; methods of estimation; food nitrogen as index of Ae.

1. Introduction

Absorption is measured as the difference between ingestion (I) and egestion (E). Absorption efficiency (Ae) is an index of the proportion of I that is transferred from the gut lumen into the body of the animal. It is usually expressed as percentage. Hence the quantitative estimation of feces has become an important component in the study of amphibian energetics. Unlike many invertebrates (e.g. Crustacea: Lautenschlager *et al* 1978), most amphibians produce semi-solid fecal pellets, which are not bagged by a 'peritrophic membrane'; on being voided, the semi-solid fecal pellets (i) lose soluble materials immediately (Marian 1982), (ii) undergo decomposition (Robinson and Bailey 1981), (iii) may be reingested by the experimental animal (e.g. *Rana catesbeiana* tadpoles: Steinwascher 1978) and/or (iv) may be ingested by the offered prey organism (e.g. *Tubifex tubifex* ingesting feces of *Rana tigrina* tadpole: Marian 1982); hence, the recovery of feces in a given experimental situation may not be complete.

Moreover, the very process of collection of feces is cumbersome and time-consuming, and yet liable for errors. Hence, relatively few estimates of Ae of amphibians have been attempted (Seale 1985). The difficulties involved in the processes of recovery and quantification of feces call for identifying an easily measurable component of food, which could serve as a reliable index of Ae of amphibians. We have considered nitrogen, a non-inert moiety of food, as a possible index of Ae and have found that the nitrogen content of food is positively and significantly correlated with the Ae of fishes, reptiles, polychaetes and aquatic insects (Pandian and Marian 1985a,b,c,d). In the present paper, we have attempted to use this approach to estimate the absorption efficiency of amphibians.

2. Materials and methods

Using gravimetric procedure (Marian and Pandian 1985), few estimates of Ae were made in the tadpoles of *Bufo melanostictus* and *Rana cyanophlyctis* (table 1). However, most information for the present study was taken from pertinent

Table 1. Absorption efficiency (Ae) of amphibians fed feed containing different nitrogen levels.

Species, size and sex	Temperature (°C)	Ration (%)	Food	Nitrogen content (% dry wt)	Absorption efficiency (%)	Comments	References
Caudates							
Plethoden cinereus	10	*ad lib*	*Lasius americanus*	7·0^a	90	T affects Ae	Merchant (1970)
	15	*ad lib*	*L. americanus*	7·0^a	86		
	20	*ad lib*	*L. americanus*	7·0^a	80		
P. cinereus	10	*ad lib*	*Drosophila* sp.	8·1^a	90	T affects Ae	Bobka *et al* (1981)
♂ ♀	15	*ad lib*	*Drosophila* sp.	8·1^a	86	but no size effect	
	20	*ad lib*	*Drosophila* sp.	8·1^a	80		
P. cinereus (0·09–0·68 g)	5, 20, 25	*ad lib*	*Drosophila* sp.	8·1^a	90	No effect of Wt and T	Crump (1979)
P. shenandoah	10	*ad lib*	*Drosophila* sp.	8·1^a	91		
	20	*ad lib*	*Drosophila* sp.	8·1^a	80		
Triturus helveticus			*Enchytraeus albidus*	9·0^a	91		Avery (1971)
T. vulgaris							
Eurycea bislineata	5	*ad lib*	Chironomid larvae	9·1^a	86	No T effect	Fitzpatrick (1973a)
	10	*ad lib*	,,	9·1^a	94		
	15	*ad lib*	,,	9·1^a	94		
Desmognathus ochrophaeus							
Gravid ♀	15	*ad lib*	*Phormia* maggots	8·0	87		Fitzpatrick (1973b)
Nongravid ♀	15	*ad lib*	*Phormia* maggots	8·0	86		
Nongravid ♀	15	*ad lib*	*Phormia* maggots	8·0	88		
Anurans							
Rana tigrina tadpole	27	*ad lib*	*Wolffia*	4·3	79	No T effect	Marian (1982)
	27	*ad lib*	*Chironomus* sp.	9·1	91		
	27	*ad lib*	*Streptocephalus*	9·0	91		
	27	*ad lib*	*Tubifex tubifex*	9·6	92		

R. tigrina tadpole	22	*ad lib*	*T. tubifex*	9·6	93	No T effect	Marian and Pandian (1985)
	27	*ad lib*	*T. tubifex*	9·6	92		
	32	*ad lib*	*T. tubifex*	9·6	94		
	37	*ad lib*	*T. tubifex*	9·6	96		
R. tigrina tadpole	27	10–100	*T. tubifex*	9·6	94	No R effect	Marian (1982)
R. tigrina (1–16 tadpoles/aquarium)	27	*ad lib*	*T. tubifex*	9·6	94	No density effect	Marian (1982)
R. tigrina tadpole (aquarium depth)	27	*ad lib*	Cabbage	9·6	94	No aquarium depth effect	Pandian and Marian (1985e)
Rana cyanophlyctis tadpole 0·56 g	26	*ad lib*	*T. tubifex*	9·6	94		Present study
R. cyanophlyctis 0·2–1 g	26	*ad lib*	*Hydrilla* sp.	4·8	73		Present study
	26	*ad lib*	*Wolffia*	4·3	74		
R. tigrina tadpole		*ad lib*	mixed diet	5·2	66		Hota (1984)
Bufo melanostictus tadpole	29	*ad lib*	carrot tuber	0·6	54		Present study
			sweet potato	1·3	51		
			beetroot	2·2	59		
			moringa leaf	4·4	71		
B. melanostictus tadpole		*ad lib*	mixed diet	5·2	70		Hota (1984)
B. terrestris adult 5–100 g	20, 25	*ad lib*	cricket	6·2*	74	No T and Wt effect	Smith (1976)
Rana tigrina adult 2–25 gW	28	3–100	*T. tubifex*	9·6	94		Marian (1982)
		ad lib	earthworm	9·1	89		
		ad lib	insect	6·3	74		
		ad lib	slug	8·0	90		

T, temperature; r, ration; Wt, body weight.

[a]Values from Campbell (1970), Nicol (1969) or estimation done in our laboratory.

publications. From a survey, about 50 publications were considered. Of them only 12 publications were finally selected; the others were not selected for following reasons: (i) lack of information of N content of the food (Altig and McDearman 1975) and (ii) reporting desired information for amphibians reared under stress conditions such as exposure to pesticide (Marian *et al* 1983). We have chosen publications reporting reliable information on total Ae (in terms of dry weight or energy) of amphibians fed on natural food under normal, healthy, experimental conditions. In some cases, it has been possible for us to secure nitrogen content of the food from other publications (e.g. *Enchytraeus*: Nicol 1969) or from our own estimation (e.g. *Drosophila*). We have made a definite effort to give due representation to herbivorous and carnivorous amphibians so that a wide range of food spectrum would be represented.

Information thus obtained has been analysed under the following headings (table 1): (i) species, body weight and sex (ii) temperature, (iii) ration, (iv) food, (v) nitrogen content of the food and (vi) Ae:

$$\text{Ae } (\%) = \frac{\text{Food absorbed}}{\text{Food consumed}} \times 100,$$

where food absorbed is $= I - E$, $I =$ food consumed, $E =$ feces egested (in terms of total dry weight or energy).

3. Results

Table 1 presents information on Ae of the tested amphibians inhabiting temperate and tropical regions. Of 11 species, for which reliable information is available, 6 are caudates and 5 are anurans; most of these caudates are adult animals and were tested on animal diet alone; the next 3 species are anuran tadpoles and were tested on a range of feeds from the nitrogen-poor sweet potato tuber to animal diet; the remaining data pertain to 2 adult anurans. The body weight of these amphibians ranged from 0·09 (*Plethoden cinereus*; Crump 1979) to 100 g (*B. melanostictus*; Smith 1976) and the nitrogen content of food from 0·6 (sweet potato tuber) to 9·6% (*T. tubifex*). Some were given different rations (3–100% of *ad libitum*) and subjected to experiments at temperature as low as 5°C and as high as 37°C. Thus, it has been possible to summarize data on the Ae of a number of amphibians studied under a wide range of feeding regimes and experimental conditions.

Figure 1 illustrates the relationship between nitrogen content of food and Ae of the tested amphibians. Tadpoles of *B. melanostictus* fed on tuber containing 0·6% N exhibited 51% efficiency; from this low value, the efficiency rapidly increased to over 90% in several amphibians fed on diet containing $> 9\%$ nitrogen. Hence the efficiency of amphibians is positively related to the nitrogen content of food.

To test whether the observed relationship is statistically significant, regression analysis is made considering nitrogen (independent variable) against Ae (dependent variable). The nitrogen content of food is positively correlated ($r = 0{\cdot}950$) to the Ae of the amphibians (figure 1); the regression obtained ($\text{Ae} = 49{\cdot}615 + 4{\cdot}596\ \text{N}$) is highly significant (F (1) 1, $39 = 354$; $p < 0{\cdot}0005$). To describe the precision of the prediction, standard error of the estimate is computed following Zar (1974), which accounts 3·748 of the total estimate. If this relationship is generally applicable, the efficiency of amphibians can reliably and precisely be predicted from the nitrogen content of food.

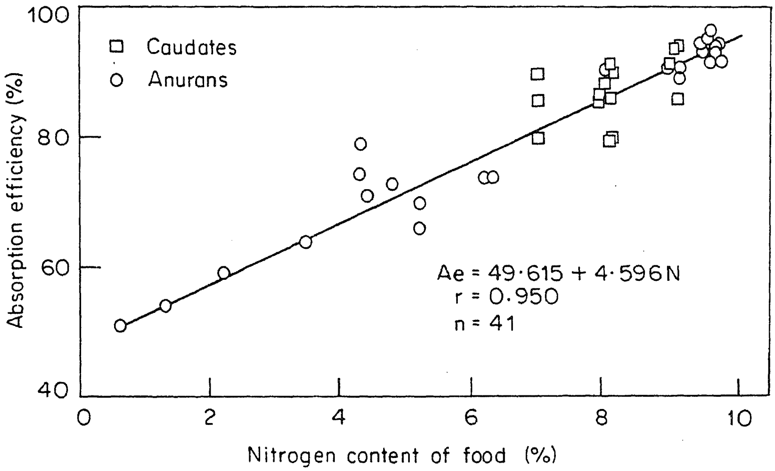

Figure 1. Relationship between the nitrogen content of food and Ae of amphibians.

4. Discussion

The conclusion that food N can precisely predict Ae of amphibians based on 41 values estimated for 11 species. Although it is based on a very few estimations of Ae, the fact that a similar relation has been observed to hold good for fishes, polychaetes, aquatic insects, lepidopterans and reptiles (Pandian and Marian 1985a,b,c,d; Pandian T J and Marian M P, unpublished results) goes to support the conclusion.

The laborious and time-consuming processes of feces recovery and quantification as well as the scope for introducing errors in the gravimetric procedure for absorption efficiency have led several investigators to defy attempts on the estimation of the efficiency of amphibians (Seale 1985). Although several direct and indirect methods (table 2) are available and frequently followed by fish biologists for estimation of Ae, amphibian biologists appear to be not aware of them. Although the rediotracer method was described by Calow and Fletcher (1972), which is relatively easier, and is based on the more sound principle, Bobka *et al* (1981) have chosen a different ^{14}C technique for the estimation of absorption efficiency using a complicated procedure. Incidentally, among the indirect procedures thus far described, the food nitrogen index method, described by Pandian and Marian (1985a), requires no quantitative recovery of feces and yet is widely applicable (Pandian and Marian 1985b,c,d; Pandian T J and Marian M P, unpublished results).

Factors such as ration and body size do not significantly influence Ae of the tested amphibian. However, there are reports for and against temperature effect on the efficiency; the efficiency of the caudates *Plethodon* spp is inversely related to temperature (Merchant 1970; Boka *et al* 1981). On the other hand, a number of workers have reported that irrespective of changes in temperature, the efficiency remains unaffected in the caudate *Eurycea bislineata* (Fitzpatrick 1973a), the toad *Bufo terrestris* (Smith 1976) and the tadpole of *R. tigrina* (Marian and Pandian 1985). An important point to be noted here is that the efficiency was estimated in these animals under a wide range of aquatic, semi-aquatic and terrestrial situations; for instance,

Table 2. Available methods for the estimation of absorption efficiency in aquatic animals.

Method	Required estimates		Nature of method	Applicability	Whether used for amphibians	Example
	Food	Feces				
1. Gravimetric	Consumption estimate required	Recovery and quantification required	Time consuming and laborious	Can be widely used	Often used	Fitzpatrick (1972a, b)
2. Artificial markers						
i) Chromic oxide	Analysis required	Recovery not required but analysis required	Requires even distribution of Cr_3O_3 in food	Applicable for dry pelleted diet only	No	Austreng (1978)
ii) Radiotracer						
a) ^{14}C	Analysis required	Recovery not required but analysis required	Requires even distribution of ^{14}C in food	Limited applicability, reliability is questionable	Rarely used	Bobka *et al* (1981)
b) ^{51}Cr	Analysis required	Recovery not required but analysis required	Requires even distribution of ^{51}Cr in food	Limited applicability	No	Calow and Fletcher (1972)
3. Natural markers						
i) Lignin	Analysis required	Recovery not required but analysis required	Time consuming	Restricted to herbivores alone	No	Klump and Nichols (1983)
ii) Ash	Analysis required	Recovery not required but analysis	Quickly done	Applicable to animals; reliability is questionable	No	Bowen (1981)
iii) Nitrogen	Analysis required	Neither recovery nor analysis required	Quickly done	Applicable to all animals	No	Pandian and Marian (1985a, b, c, d)

Marian and Pandian (1985) estimated the efficiency of *R. tigrina* tadpoles kept in aquarium. Bobka *et al* (1981) made the estimate of *Plethodon* maintained in moist terrarium. It is apparent that when the efficiency of an amphibian is estimated in an aquarium, temperature fails to affect the efficiency but when it is estimated in a terrarium, the reverse is true. Therefore, water appears to have a modifying effect on the simple linear relationship observed between food-N and Ae. It is known that the simple linear relation between food-N and Ae is secondarily modified by water content of food in lepidopterans and other arthropods (Pandian T J and Marian M P, unpublished results). In general, terrestrial animals acquire water mainly from food, and gain or lose water through the body surface (Tracy 1976) and hence are forced to acquire more water by accelerating the alimentation process; such an acceleration will lower the efficiency (Ae). Pandian and Marian (unpublished results) have shown that the nitrogen is more important in the prediction of Ae, and water can serve as co-predictor to improve the accuracy of prediction of Ae from food-N. Basic data on the effect of water on Ae are urgently required for more amphibians, before it could be considered as a co-predictor. However, food-N, can precisely predict Ae of aquatic amphibians, in which feces recovery and quantification are difficult. In figure 2, a comparison of the trends obtained for food-N and Ae relation in fishes (Pandian and Marian 1985a), amphibians and reptiles (Pandian and Marian 1985b) are made; a positive and significant correlation observed between food-N and Ae of these animal groups renders it possible to predict Ae from food-N with more than 90% accuracy. It may be noted that the given diet is more efficiently digested and absorbed by amphibians than by fishes and reptiles; for instance, fed on food containing 6% N, the efficiency is 78% for amphibians in comparison to 57 and 63% for fishes and reptiles, respectively. The reason for this dissimilarity in the efficiency among these animal groups are not clear at present; further work and analysis are in progress to know the underlining physiological processes that is responsible for the differences.

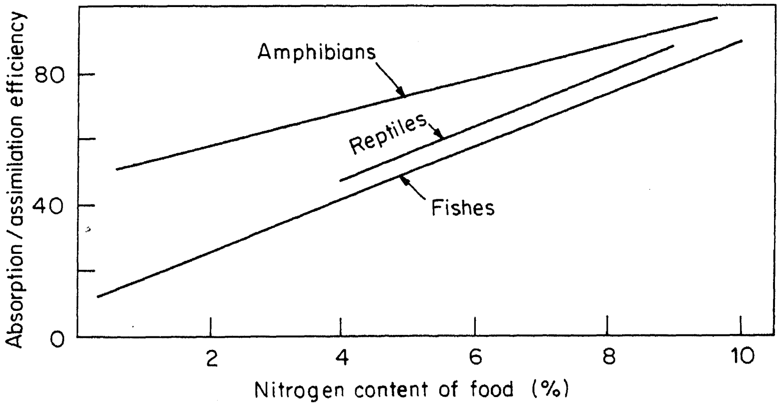

Figure 2. Comparison of the trends obtained for food–N and absorption/assimilation efficiency relation in fishes, amphibians and reptiles. For fishes ($\log Ae = 1{\cdot}3706 + 0{\cdot}5807 \log N$) and reptiles ($Ase = 14{\cdot}424 + 8{\cdot}210\,N$). Values are taken from Pandian and Marian (1985a, b).

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Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita*

Ch VENKATA RAO**, A MANI* and P KAMESWARA RAO
Division of Entomology and *Citrus Improvement Project, SV Agricultural College, Tirupati 517 502, India
**Present address: Office of the Director of Agriculture, Eluru 534 002, India

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Abstract. Thirteen leaves, 4 oilcake extracts and 4 root exudates were tested against root-knot nematode, *Meloidogyne incognita* to study their effect on egg hatch and larval mortality. Aqueous leaf extracts of *Datura stramonium*, *Parthenium hysterophorus* and *Tagetes erecta* and oilcake extracts of karanj and neem were the most potent treatments and they not only inhibited egg hatch but also killed the larvae significantly. The root exudates of *Brassica campestris* and *Tagetes erecta* also killed the second stage larvae of *Meloidogyne incognita*.

Keywords. Plant products; toxicity; *Meloidogyne incognita*.

1. Introduction

In vitro experiments carried out earlier revealed that certain aqueous extracts of dried leaves and oilcakes inhibited egg hatch and larval mobility of *Meloidogyne incognita* (Kofoid and White 1919), Chitwood 1949 (Gowda and Setty 1972; Khan *et al* 1975; Mishra and Prasad 1975; Nath *et al* 1982). The effect of root exudates of marigold, *Tagetes* spp., in reducing populations of *Pratylenchus penetrans* and *Tylenchorhynchus* spp. had been reported by Oostenbrink (1961). There is potential scope for the use of plant products in nematode control as they are cheap, easily available and non-toxic to mammals. An attempt was made to test the effect of aqueous extracts of a few dried plant leaves, oil cakes and root-exudates on egg hatch and larval mortality of *M. incognita*.

2. Materials and methods

Leaves of 13 plant species and 4 non-edible oil cakes were collected, dried under shade and powdered well. Standard extract was prepared by grinding 10 g of powdered leaf or oil cake with 50 ml of distilled water using pestle and mortar and passing through double layer of muslin cloth. The standard extract was diluted to 5% and it was transferred into 5 cm diameter petri dishes at 5 ml per dish. Five uniform size egg masses of *M. incognita*, handpicked from infected brinjal roots, were kept in each petri dish for hatching. Tap water served as check and each treatment was replicated 3 times. All the petri dishes were maintained at 26 ± 2°C in BOD incubator. Observation on egg hatch was recorded at 3 days interval and egg masses from all the treatments were transferred to tap water after 15 days of exposure in extracts. Hatch in water was recorded upto 30 days.

The effect of leaf and oil cake extracts on larval mortality was tested at two concentrations viz 1 and 5% prepared from the standard extracts. Five ml of the extract was pippetted out into 5 cm diameter petri dishes and 100 freshly hatched second stage larvae were suspended in each dish. Observation on larval mortality was recorded after 12, 24 and 48 hr of exposure. The mortality of larvae was confirmed by gently touching them with a fine needle.

Four test plants viz marigold (*Tagetes erecta*) periwinkle (*Vinca rosea*), gingelly (*Sesamum orientale*) and mustard (*Brassica campestris*) were grown separately in earthen pots at 5–7 plants per pot. Root exudates were collected from 60 days old test plants (Southey 1970), filtered using Whatman No. 42 filter paper and tested against second-stage larvae of *M. incognita* as mentioned earlier.

The per cent larval mortality recorded in treatment was calculated and corrected according to Abbott's (1925) formula to eliminate the mortality observed in check. Data were analysed following standard procedures for analysis of variance. Differences between means were evaluated for significance according to Duncan's multiple range test (Steel and Torrie 1960). All differences referred to in the text were significant at 5% level of probability.

3. Results and discussion

A marked reduction in hatching of *M. incognita* eggs was observed in all the leaf extracts tested (table 1). The inhibition of egg hatch in leaf extracts of *Parthenium hysterophorus*, *T. erecta* and *Datura stramonium* was higher than in other extracts

Table 1. Effect of leaf and oil cake extracts on hatching of *M. incognita* eggs*.

Treatments	Egg hatch in extract (15 days)	Total hatch (15 days in extract + 15 days in water)	Inhibition of hatch over check (%)
Leaf extracts			
Check (water)	1248 i	1538 e	—
A. indica	753 de	968 c	37·06
Calotropis gigantea	908 d	1461 e	5·00
D. stramonium	291 b	691 b	55·07
Eucalyptus globulus	1156 hi	1486 e	3·38
Glyricidia maculata	339 c	817 c	46·87
Ipomea cornea	962 fg	1524 e	0·91
Leucaemea leucophyla	645 cd	1394 de	9·36
Nerium indicum	509 c	959 c	37·64
Pongamia glabra	717 d	1509 e	1·88
Prosopis juliflora	961 fgh	1437 de	6·56
P. hysterophorus	110 a	457 a	70·28
Sapindus emerginatus	1154 ghi	1490 e	3·12
T. erecta	326 b	690 b	55·13
Oil cake extracts			
Castor cake	1104 ghi	1496 e	2·73
Gingelly cake	889 ef	1536 e	0·13
Karanj cake	510 c	1015 c	34·00
Neem cake	565 c	1176 cd	23·53

Mean of 3 replications

*Data in columns followed by a common letter were not statistically different ($P = 0{\cdot}05$) according to Duncan's multiple range test.

and the inhibition was 70·28, 55·13 and 55·07%, respectively. However, the leaf extract of *P. hysterophorus* was the most effective treatment wherein the highest inhibition was recorded. Hussain and Masood (1976) reported complete inhibition of hatching of *M. incognita* eggs in certain leaf extracts like *Azadirachta indica* and *Chenopodium anthelminticum.* Among oil cake extracts, karanj and neem cake extracts inhibited egg hatch to the extent of 34·00 and 23·53%, respectively. Similarly Khan *et al* (1975) reported that egg hatch of *M. incognita* was suppressed by aqueous extracts of mahua and neem cakes. But in the present study it was observed that in extracts of castor and gingelly cakes, egg hatch was almost normal. This is in variance with the findings of Khan *et al* (1975) which could be due to the difference in the method of preparation of the extracts as well as the concentration used.

With regard to larval mortality, all the leaf and oil cake extracts were toxic to the second stage larvae (tables 2 and 3). An increase in the concentration of extracts as well as the exposure period resulted in a corresponding increase in larval mortality. However, a high rate of larval mortality was recorded at 5% concentration after 48 hr of exposure. The extract of *P. hysterophorus* was the most potent among leaf extracts and was on par with the extracts of *D. stramonium* and *T. erecta.* The larval mortality was maximum in neem cake extract followed by those of karanj, gingelly and castor cakes. Mishra and Prasad (1975) reported that 1% solution of neem cake extract was highly toxic to larvae of *M. incognita.* The adverse effect of plant products in suppressing nematode activities like egg hatch and larval mobility could be due to the presence of toxic principles like alkaloids, cyanogenic glycosides, glycosides, phenols, terpenoids etc.

Present investigations further revealed that all the 4 root exudates significantly increased the larval mortality with increase in observation period (table 4). The highest larval mortality of 59·32% was recorded after 48 hr of exposure in the root exudate of

Table 2. Effect of leaf extracts on larval mortality of *M. incognita.**

	1% Extract			5% Extract		
	Mortality at different time intervals (hr) (%)			Mortality at different time intervals (hr) (%)		
Treatments	12	24	48	12	24	48
A. indica	8·63 b	11·54 cdef	15·48 cde	17·60 cdefg	23·02 de	32·31 cd
C. gigantea	15·99 a	24·16 ab	29·14 ab	25·16 abcd	33·17 bcd	46·45 b
D. stramonium	21·22 a	23·08 ab	33·49 a	25·37 abc	38·91 ab	59·60 a
E. globulus	3·62 cdef	1·19 g	10·57 e	6·05 hij	10·68 f	17·72 f
G. maculata	3·05 ef	9·07 cfg	12·79 cde	5·05 ij	19·34 ef	20·02 ef
I. cornea	15·38 a	15·97 bc	20·93 bcd	15·85 defgh	23·05 de	24·80 def
L. leucophyla	3·05 ef	3·81 fg	9·62 e	11·46 fghi	22·89 e	28·52 cdef
N. indicum	8·59 bc	12·95 cd	22·55 bc	20·65 bcde	24·24 cde	33·37 cd
P. glabra	3·95 bcdef	10·07 defg	14·82 cde	13·92 efghi	26·90 cd	36·05 c
P. juliflora	5·92 bcd	11·08 cdef	22·90 bc	9·47 ghij	18·79 ef	30·32 cde
P. hysterophorus	20·14 a	27·18 a	36·76 a	32·46 a	45·85 a	65·00 a
S. emerginatus	4·65 bcde	11·79 cde	18·56 cde	17·93 bcdef	23·38 de	34·18 cd
T. erecta	20·48 a	21·89 ab	28·97 ab	25·95 ab	34·78 bc	59·50 a

Mean of 3 replications

*Data in columns followed by a common letter were not statistically different ($P=0{\cdot}05$) according to Duncan's multiple range test.

Table 3. Effect of oil cakes extracts on larval mortality of *M. incognita.**

	1% Extract			5% Extract		
	Mortality at different time intervals (hr) (%)			Mortality at different time intervals (hr) (%)		
Treatments	12	24	48	12	24	48
Castor cake	12·11 a	13·95 ab	13·64 b	18·60 b	20·75 b	21·60 b
Gingelly cake	2·93 a	17·60 ab	19·14 ab	12·68 b	18·64 b	22·14 b
Karanj cake	3·57 a	7·27 b	13·74 ab	18·33 b	21·26 b	25·33 b
Neem cake	15·89 a	21·90 a	23·64 a	32·99 a	53·52 a	57·00 a

Mean of 3 replications

*Data in columns followed by a common letter were not statistically different ($P = 0{\cdot}05$ according to Duncan's multiple range test.

Table 4. Effect of root exudates on larval mortality of *M. incognita**.

	Mortality at different time intervals (hr) (%)		
Treatment	12	24	48
B. campestris	16·07 a	24·48 b	42·80 b
S. orientale	9·55 a	19·36 b	27·07 c
V. rosea	16·52 a	24·77 b	36·17 bc
T. erecta	18·88 a	53·56 a	59·32 a

Mean of 3 replications

*Data in columns followed by a common letter were not statistically different ($P = 0{\cdot}05$) according to Duncan's multiple range test.

T. erecta followed by 42·80, 36·17 and 27·07% in root exudates of *B. campestris*, *V. rosea* and *S. orientale*, respectively. Oostenbrink *et al* (1961) reported similar effect of root exudate of *Tagetes* spp. on *P. penetrans* and *Tylenchorhynchus* spp. The toxic effect could be due to the release of certain toxic metabolites from roots. Bakker and Gommers (1978) reported that the root exudate of *Tagetes* spp. contained compounds like α-terthienyl which could have affected nematode activities.

4. Conclusions

Application of organic amendments such as plant leaves and oil cakes is a common practice for nematode management (Singh and Sitaramaiah 1973; Khan *et al* 1974). The present investigations reveal that there is potential scope for utilizing several plant products in nematode control. However, further research should be carried out to determine the efficacy of such plant products in controlling various plant parasitic nematodes. Likewise, plants which release toxic root exudates may be grown as intercrops or mixed crops and they can also be included in crop rotation depending on the regional cropping pattern for nematode management.

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Annual variation in the oxygen utilisation dynamics of the garden lizard, *Calotes versicolor*

B B P GUPTA* and J P THAPLIYAL*
Department of Zoology, Banaras Hindu University, Varanasi 221 005, India
*Present address: Department of Zoology, School of Life Sciences, North-Eastern Hill University, Nongthymmai, Shillong 793 014, India

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Abstract. Energy requirement of *Calotes versicolor*, as judged by the rate of oxygen consumption by whole body, liver and muscle, was recorded to be minimum during hibernation (quiescent phase) and maximum during breeding phase. Kidney metabolic rate was comparatively high during hibernation and post-hibernation. Statistically significant circannual rhythms were detected in the rate of oxygen consumption of whole body, liver, muscle and kidney but not in that of brain. However, significant but transient increase in the rate of brain oxygen uptake was recorded during February (arousal), May (initiation of breeding) and November (entry into hibernation). It seems that the annual changes in metabolic rate are reflections of energy requirements of the lizard and its tissues during different phases of the annual activity cycle. Further, climatic factors seem to affect the oxidative metabolism of *Calotes* acting through the endocrine glands.

Keywords. Reptiles; energy metabolism; respiration; oxygen consumption; hibernation; reproduction.

1. Introduction

There are various reports on physiological and biochemical reflections of circannual rhythmicity in homeotherms (Johansson and Senturia 1972; Senturia and Johansson 1974), however, information available on poikilotherms is scanty (Bennett and Dawson 1976; McDonald 1976). While information on behavioural thermoregulation and on the physiology of activity in lizards is well documented (Bennett 1973a,b, 1980; Bennett and Dawson 1972; Huey and Slatkin 1976; Avery 1976), little attention has been paid towards the energetics for reproduction and hibernation (McDonald 1976; Bennett and Dawson 1976). Unlike in temperate zone reptiles, little is known about the physiological correlates of metabolically critical annual events like hibernation, molting and reproduction in tropical reptilian species (McDonald 1976). Earlier reports were concerned mainly either with the whole body oxygen consumption or tissue respiration of lizards during and/or after hibernation. However, no attempt has been made to investigate the sequential changes simultaneously in both the whole body oxygen consumption and tissue respiration during different phases of the annual activity cycle, and especially in relation to the ever-changing both internal and external factors which are known to influence the over-all metabolism of reptiles (Bennett and Dawson 1976; McDonald 1976; Thapliyal 1980a,b; Gupta 1982). Most of the reports are confined to the energy metabolism of temperate species only (Bennett and Dawson 1976; Thapliyal and Gupta 1984). Moreover, reports on seasonal variations in the rate of tissue respiration is limited to two species only, i.e., *Thamnophis sirtalis* (Hoskins and Aleksiuk 1973) and *Natrix piscator* (Thapliyal and Sharan 1980). These studies were limited to few months only, and no attempt was made to study circannual variations in whole body oxygen consumption. Therefore, an attempt was made to study the annual changes in whole body oxygen consumption and the rate of tissue respiration especially in relation to reproduction and hibernation in a tropical lizard, *Calotes versicolor*.

2. Materials and methods

C. versicolor is a small-sized, non-poisonous, carnivore lizard found throughout the year in Varanasi (latitude, 25°18′ N; longitude, 83°01′ E). In nature as well as in laboratory conditions *C. versicolor* hibernates during winter months and breeds during monsoon. The annual cycle of activity of the lizard at Varanasi can be divided into 4 distinct phases: hibernation (November–January), post-hibernation (February–May), breeding (June–August), and pre-hibernation (September–October).

Adult male *C. versicolor* (average body weight 30 ± 2 g; snout to vent length 10 ± 2 cm) were collected from area during the first week of each month and were caged in wire-netted wooden cages fully exposed to environment: natural day length, temperature and other climatic factors. Animals were acclimatized for one week. During this period they were provided with live maggots (meal worms) and water *ad libitum*. At the end of 7 days, whole body oxygen consumption of 4 lizards was measured at 30°C in each month using a constant pressure volumetric respirometer under complete darkness (McDonald 1976).

Animals were fasted for 24 hr before measuring the rate of respiration. Further, to minimise the effect of circadian changes on the rate of oxygen consumption (Pati *et al* 1982), the rate of respiration was always measured 2 hr before sunset. A 30 day interval was maintained between subsequent measurements. Just after the measurement of whole body oxygen consumption, lizards were decapitated, tissues (liver, skeletal muscle, kidney and brain) removed, rinsed in ice-cold reptilian Ringer's solution (Gupta 1982) and stored in a freezer at −15°C. The rate of tissue respiration was measured in a Warburg apparatus (Warburg Apparat V166; B Braun Melsungen, Federal Republic of Germany) following the method of Umbreit *et al* (1964). α-Ketoglutaric acid (30 mM) was used as substrate (for details see Gupta and Thapliyal 1983). Results were expressed in ml O_2/g/hr and μl O_2/mg wet tissue/hr for the rate of oxygen consumption by whole body and tissue respiration, respectively.

The mean environmental temperature was measured at the cage level (figure 1). Data were analysed statistically with the help of Student's 't' test (Snedecor 1961). For evaluating the circannual rhythms in 12 months data, least square fitting of cosine function was utilized (Halberg *et al* 1972).

3. Results

An annual pattern of variation in the rate of oxygen consumption by whole body of the lizard and its tissues was recorded (figure 1). While the rate of oxygen consumption of whole body, liver and muscle was minimum during hibernation, it increased gradually during post-hibernation and reached a maximum during the breeding phase. The metabolic rate of kidney and brain were comparatively more during hibernation and were greater during the post-hibernation period (table 1). Further, a significant

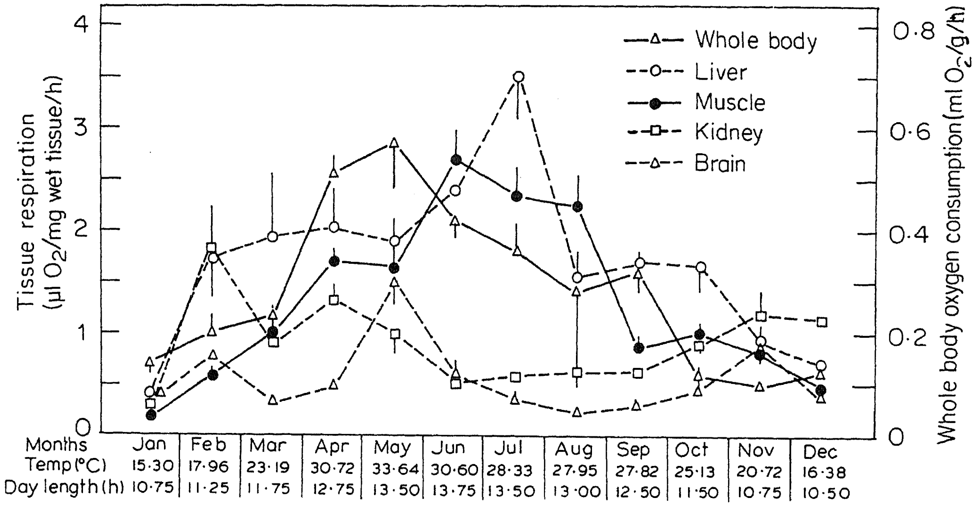

Figure 1. Annual variations in the rate of oxygen consumption by whole body and isolated tissues (liver, muscle, kidney and brain) of *C. versicolor*. Vertical bars represent 1 Standard error ($n=4$).

Table 1. Average metabolic rates of whole body and different tissues during different phases of the annual activity cycle of *C. versicolor*.

Variable (Unit)	Hibernation ($n=12$)	Post-hibernation ($n=16$)	Breeding ($n=12$)	Pre-hibernation ($n=8$)
Whole body oxygen consumption (ml O_2/g/h)	0·12±0·01*	0·38±0·05^b	0·35±0·02^b	0·22±0·04^{a,c}
Tissue O_2 consumption (O_2/mg wet tissue/h)				
a) Liver	0·64±0·09	1·90±0·20^b	2·42±0·31^b	1·67±0·11^{b,c}
b) Muscle	0·48±0·09	1·25±0·14^{b,d}	2·47±0·16^b	0·94±0·07^{b,d}
c) Kidney	0·92±0·13	1·29±0·14^d	0·58±0·05^a	0·77±0·06^c
d) Brain	0·55±0·08	0·76±0·12^c	0·39±0·06	0·38±0·05

*Mean ± SE

^{a,b}Differ from the value of hibernation: $P<0·05$ and $P<0·001$, respectively.

^{c,d}Differ from the value of breeding: $P<0·05$ and $P<0·001$, respectively.

Based on the data plotted in figure 1.

transient increase in the brain O_2 uptake was found during the months of February, May and November (figure 1).

When the data were analysed for circannual rhythm test with the help of least square fitting of cosine function, significant circannual rhythms were detected in the rate of oxygen consumption by whole body, liver, muscle and kidney (table 2, figure 2). However, no significant circannual rhythm was recorded for brain oxygen consumption. While acrophases for metabolic rate of whole body, liver and muscle were obtained during breeding phase, that of kidney was recorded during February—the period of arousal from the winter sleep (table 2, figure 2).

Table 2. Rhythmometric summary based on least-square fitting of 365·25–d cosine function (365·25 = 360°) to data plotted in figure 1.

Variable (Unit)	Key to elipses	No. obs.^{a}	PR^b	P^c	M^d ± SE^e	A^f ± SE	ϕ^g ± SE (95% CL^h)	
							Degree	Month/date
Whole body oxygen consumption (ml O_2/g/h)	A	48	67	0·001	0·281 ± 0·014	0·197 ± 0·020	−205 ± 06 (−193; −217)	July 14 (July 2; July 26)
Tissue O_2 uptake (μl O_2/mg wet tissue/h)								
Liver	B	48	41	0·001	1·679 ± 0·111	0·873 ± 0·158	−186 ± 10 (−166; −206)	June 25 (June 5; July 15)
Muscle	C	48	73	0·001	1·298 ± 0·065	1·023 ± 0·092	−182 ± 05 (−172; −192)	June 21 (June 11; July 1)
Kidney	D	48	21	0·005	0·933 ± 0·065	0·313 ± 0·092	−47 ± 17 (−14; −80)	Feb. 7 (Jan. 5; March 11)
Brain	E	48	9	0·129	0·552 ± 0·054	0·159 ± 0·077	−110 ± 28 (−0; −0)	April 10 (00; 00)

[a] Number of observations; [b] Percent rhythm (percentage of variability accounted for by cosine curve); [c] Probability of hypothesis that amplitude = 0; [d] Mesor (arithmatic mean of observations made over 12 months); [e] Standard error; [f] Amplitude (one half difference between highest and lowest values); [g] Acrophase (lag between the timing of peak metabolic function and arbitrary reference time (Ref., December 22); [h] Confidence limit.

See figure 2 for elipses.

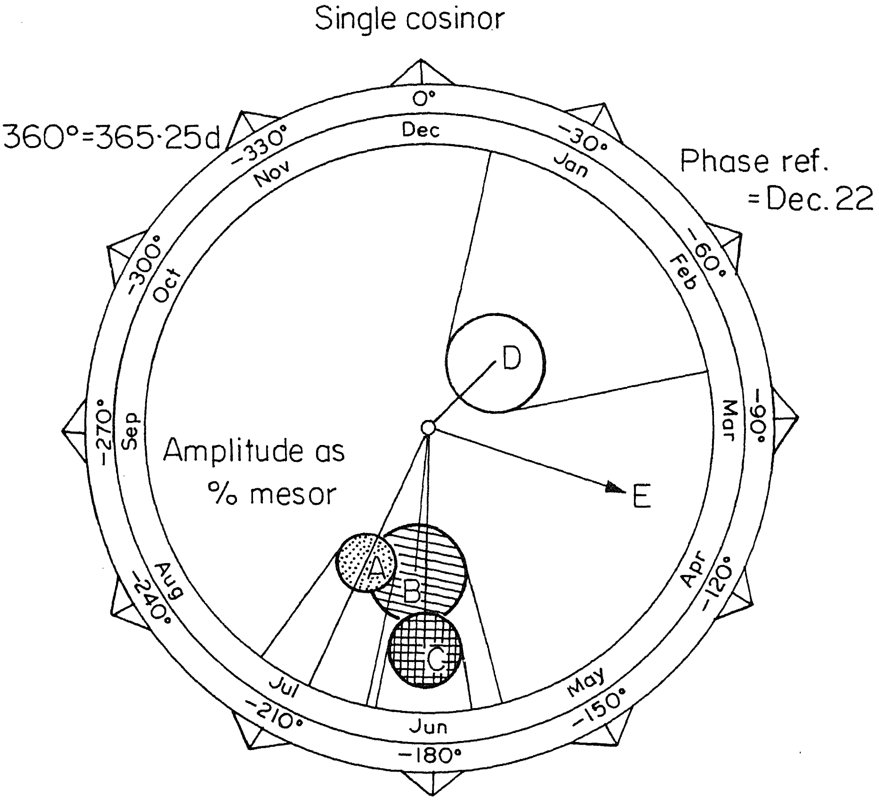

Figure 2. Microscopic parameter estimations on circannual rhythms of oxygen consumption rate by whole body and different tissues of *C. versicolor*. The direction of the vector and the area covered by two lines which touch the inner circle at the periphery indicate the span of greater metabolic activity. The length of the same vector indicates the extent of predictable periodic changes, i.e., the rhythm amplitude (expressed as % mesor). The circle does not overlap the pole indicates detection of rhythm by cosinor method. Calculation is based on least square fitting of 365·25 d cosine curve. (**A**), Whole body; (**B**), liver; (**C**), muscle; (**D**), kidney; (**E**), brain.

4. Discussion

The present study is a comprehensive study of the annual variation in the rate of oxygen consumption of a tropical reptile and of its vital tissues (liver, muscle, kidney and brain) during the year.

Seasonal variations in the rate of whole body oxygen consumption indicate that the metabolic rate of *C. versicolor* changes with the energy demand during different phases of the annual activity cycle. During hibernation, when most of the metabolic pathways are either non-operative or running at a low level, and endocrine glands (except chromaffin tissues) are inactive or minimally active (Singh 1968; Thapliyal and Chandola 1973; Kashinathan and Basu 1973; Haldar 1977; Thapliyal 1980b), the lizard becomes lethargic and inactive, and needs minimum energy just sufficient for the maintenance of life. During this phase the metabolic rate of the lizard is not influenced by high ambient temperature even when raised artificially (Gupta and Thapliyal 1985; Thapliyal 1980b). Further, monthly changes in whole body O_2

consumption and tissue respiration exhibit no direct relationship with the monthly variation in the ambient temperature (figure 1). Gradual increase in the metabolic rate during post-hibernation seems to indicate an increased capacity to meet the enhanced energy requirements for the increased activity associated with feeding/foraging, offence/defence etc. During the breeding phase the metabolic rate of the lizard remained fairly high probably due to a further increase in energy demand for the maintenance of vigorous activity related to reproductive functions. Higher metabolic rate during pre-hibernation than that during hibernation may be due to high energy demand for post-nuptial molting (figure 1). It is thus obvious that the metabolic rate of the lizard depends upon the need of energy production during different phases of the annual activity cycle.

The annual patterns of oxygen consumption of the tissues were not similar. While changes in the oxidative capacity of liver seem to be correlated with the availability of food and feeding behaviour, the oxidative capacity of muscle is correlated with the level of activity of the lizard during different months of the year (figure 1). The higher rate of kidney oxygen consumption in mammals is reportedly associated with energy production required for conservation of water and electrolytes, and excretion of harmful metabolites (Kill 1971). Thus, the high metabolic rate of kidney during hibernation seems to indicate high rate of energy production. The increased kidney O_2 uptake during hibernation, when the lizard experiences prolonged cold stress, may be of a great physiological significance. Since the presence of excretory products (i.e., urea, uric acid, and other nitrogenous waste products) in blood even in minute quantity may endanger survival of the lizard during hibernation. Therefore, kidney probably requires more energy to meet the excretory demand. High oxidative capacity of brain during winter months seems to indicate increased activity of the brain. In *C. versicolor* as well as in other reptiles, brain oxygen consumption has been shown to be increased under artificially lowered room temperatures (Das and Patnaik 1979; Sharan *et al* 1981). It thus seems that the increase in the central oxidative capacity in response to low temperatures of winter protects the animal against freezing during hibernation phase. Transient increase in the metabolic capacity of brain during February (month of arousal), May (when *Calotes* enters in breeding phase) and November (time to enter hibernation) may be associated with the increased synthesis of chemical transmitters and/or transmission of neural impulses which are critical for signalling critical time for initiation and termination of reproduction and hibernation (Thapliyal and Sharan 1980). The high metabolic capacity may also be required to meet the increased energy demand of the brain tissue to maintain ion gradients for high electrical activity during these critical periods.

Statistically significant (table 2) circannual rhythms in the metabolic rate of whole body, liver and muscle with acrophases during breeding phase indicate that, to meet greater energy demand during breeding phase, annual cycles in metabolic rate and reproductive functions are synchronized by some common factor(s). Acrophase of Kidney O_2 uptake during February may be associated with sudden acceleration of catabolic processes at the time of arousal. The absence of a significant circannual rhythm in brain tissue respiration seems to suggest a constant level of brain activity throughout the year, and also its independence from the annually changing factors, both internal and external. Obviously, the stabilized and self-regulated metabolic capacity of brain probably helps the lizard in co-ordinating vital metabolic processes for successful survival under the high summer and low winter temperatures.

Acknowledgement

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Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina*

J MUTHUKRISHNAN, M SENTHAMIZHSELVAN and S MOHAN
Department of Zoology, APA College, Palni 624 602, India

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Abstract. Activity of tail, gut and muscle acid phosphatase of *Rana tigrina* tadpoles in relation to metamorphic index and temperature was studied. Optimum pH of the enzyme was between 4·0 and 4·5. K_m for the muscle phosphatase was low, indicating its greater affinity to the substrate. With advancing stage of metamorphosis or increasing temperature, activity of the enzyme increased. At metamorphic climax the activity was low. Tail acid phosphatase displayed higher activity than the gut or muscle phosphatase, indicating the role of the enzyme in autolysis of the tail during metamorphosis.

Keywords. Acid phosphatase; metamorphic index; *Rana tigrina*.

1. Introduction

Tadpoles of *Rana tigrina* are subjected to variations in temperature, availability of food and depth of water column, which significantly alter the rate of metamorphosis (Marian and Pandian 1985). Acid phosphatase which is a lysosomal enzyme, plays an important role in autolytic degradation of tissues such as the regression of tail during metamorphosis of tadpoles (Nath and Butler 1971). Locke and Collins (1968) and Collins (1975) suggested that acid phosphatase can be used as an index of lysosomal activity. Acid phosphatase is also associated with growth and differentiation of tissues (Barker and Alexander 1958). Muthukrishnan and Senthamizhselvan (1985) showed that acid phosphatase plays an important role in the utilization of yolk during the embryonic development of the dragonfly *Mesogomphus lineatus*. Marian and Pandian (1985) concluded that temperature is a potent factor which significantly influences the rate of metamorphosis of *R. tigrina* tadpoles. Therefore, temperature is likely to influence acid phosphatase activity. The present paper reports on acid phosphatase activity of *R. tigrina* tadpoles as a function of metamorphic index (MI) and temperature.

2. Materials and methods

Acid phosphatase activity of the chosen tissues was estimated following the method described by Bergmeyer (1963). *p*-Nitrophenol (PNP) phosphate was used as the substrate. Three series of experiments were carried out. In the first series, optimum pH, and K_m for the tail, gut and muscle acid phosphatase of the tadpole (fore-limb bud stage, reared at 27°C) were determined. In the second series, activity of the enzyme from the tail, gut and muscle of the tadpoles of different MI reared at 27, 32 and 35°C was estimated. In the third series, multiplicity of the enzyme was tested electrophoretically. For the estimation of acid phosphatase activity, an incubation mixture consisting of 0·1 ml of the enzyme extract (the supernatant of 10%

homogenate (w/v) of the chosen tissue in distilled water, centrifuged at 1000 g for 10 min), 0·5 ml of substrate ($6{\cdot}25 \times 10^{-4}$ M) and 0·5 ml of acetate buffer (0·2 M) was used. The reaction mixture was incubated for 30 min at the temperature (27, 32 or 35°C) at which the tadpoles were acclimated. Activity of the enzyme was expressed in units of mg PNP released/mg protein/hr. Protein content of the enzyme was estimated following the method of Lowry *et al* (1951). For the electrophoretic separation of the enzyme, a polyacrylamide disc gel (7%) was used. 0·03 M Tris-HCl (pH 8·9) and 0·02 M Tris-glycine (pH 8·4) were used as gel and tank buffers, respectively. About 0·5 ml (approx. 250 μg) protein of the enzyme extract was loaded into the gel tubes. A constant current of 3 mA was supplied. The gel was stained in 1-naphthyl phosphate and fast blue RR at the optimum pH (4·0 or 4·5) and incubated at 37°C for 15 min.

3. Results

3.1 *Optimum pH and K_m*

Acid phosphatase of *R. tigrina* displayed the highest activity at 4·5 pH (tail) or 4·0 pH (gut and muscle) (figure 1). K_m for the enzyme from tail, gut and muscle were $2{\cdot}78 \times 10^{-5}$ M, $3{\cdot}75 \times 10^{-5}$ M and $2{\cdot}38 \times 10^{-5}$ M, respectively. Therefore, the muscle acid phosphatase appears to have more affinity to the substrate than either the tail or gut phosphatase.

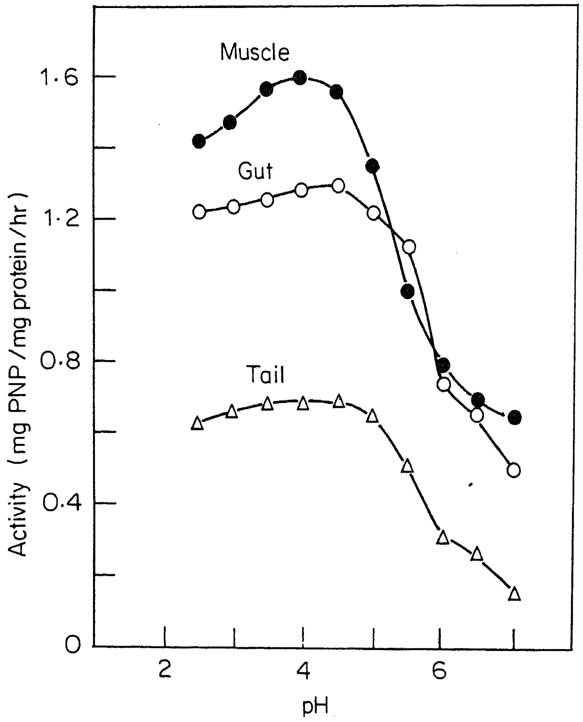

Figure 1. Activity of tail, gut and muscle acid phosphatase of *R. tigrina* tadpole as a function of pH.

3.2 *Effect of MI*

After reaching the fore-limb bud stage, the tadpoles reared at 27, 32 and 35°C required 8·0, 5·0 and 3·0 days, respectively to complete metamorphosis (figure 2). With advancing metamorphic period, activity of the enzyme from the tested tissues increased and at the metamorphic climax, it declined slightly. For instance, at 27°C, activity of the tail phosphatase increased from 0·562 mg PNP/mg/hr for the tadpoles of 2·523 MI to 1·197 unit for those of 1·133 MI; it decreased to 1·02 unit in the tadpoles at metamorphic climax. Similar trends were obtained for the gut and muscle phosphatase (figure 3). The stage at which the enzyme displayed maximum activity varied with temperature. For instance, while the peak activity of the tail acid phosphatase was observed in the tadpoles of 1·333 MI at 27°C, at 32 and 35°C it was observed at 1·13 MI (figure 3). At 32°C, tail, gut and muscle phosphatase activity was highest in the tadpoles belonging to 1·13 and 1·04 MI and freshly metamorphosed adult, respectively (figure 3B). At 35°C gut and muscle phosphatase displayed peak activity at a very early stage (1·909 MI), while the tail phosphatase displayed maximum activity only at a later stage (1·13 MI) (figure 3C).

3.3 *Effect of temperature*

Irrespective of the source of the enzyme, the activity increased with increasing temperature. Increase in temperature from 27–32 and 35°C, increased the activity of

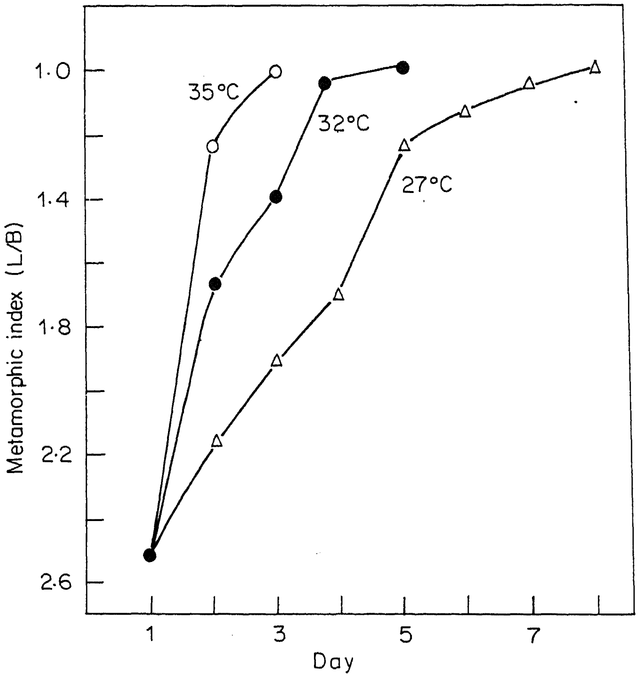

Figure 2. Metamorphic index of *R. tigrina* tadpole reared at the tested temperature as a function of age.

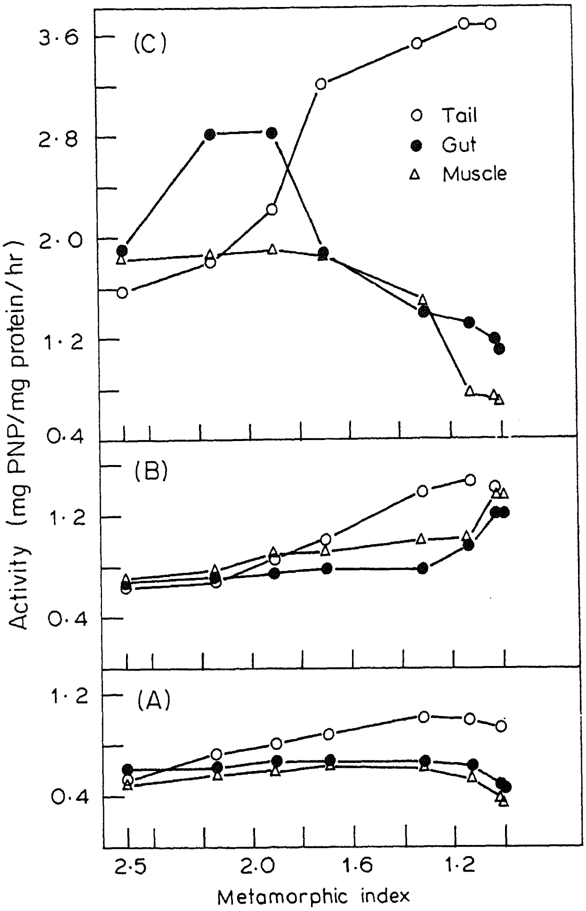

Figure 3. Activity of tail, gut and muscle acid phosphatase of *R. tigrina* tadpole as a function of MI. Panels **A**, **B** and **C** pertain to the activity at 27, 32 and 35°C, respectively. Each value is the mean of 3 estimations.

the tail phosphatase of 1·33 MI tadpoles from 1·197–1·42 and 3·56 units (figure 3). Influence of temperature of gut and muscle acid phosphatase was slightly different from that of the tail phosphatase. For instance, increase in the gut phosphatase activity of 1·33, 1·13 and 1·04 MI tadpoles due to increase in the temperature from 27–35°C was less than that for the other stages (figure 3). The response of muscle acid phosphatase to increase in temperature was quite different from that of the tail or gut phosphatase. In tadpoles of 1·13 and 1·04 MI, the enzyme activity decreased at 35°C, while, for all the other stages the activity increased (figure 3).

3.4 *Enzyme multiplicity*

Electrophoretic separation of the enzyme revealed the presence of 5, 4 and 3 fractions in the extracts from the muscle, tail and gut, respectively (figure 4).

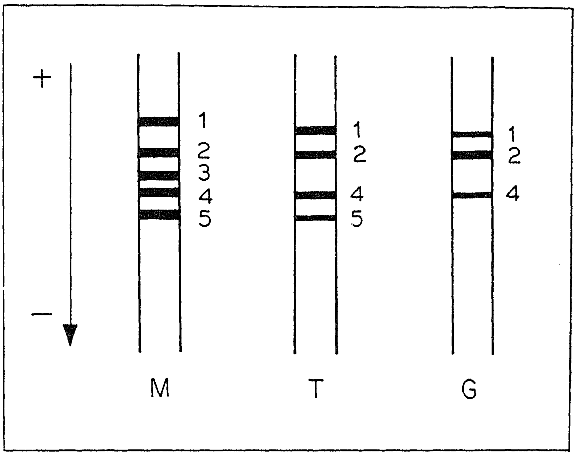

Figure 4. Electrophoretograms of muscle (M), tail (T) and gut (G) acid phosphatase of *R. tigrina* tadpole.

4. Discussion

The optimum pH of (4·0 and 4·5) reported in the present study agrees with that for the isopod *Porcellio leavis* (Saleem and Alikhan 1974). Generally the enzyme is reported to display maximum activity between 15–30°C but in the present study, temperature accelerated the activity of the enzyme to different levels. The finding that tail phosphatase activity was extremely high at 35°C may be attributed to the shortening of the metamorphic period to 4 days at the temperature. With advancing stage of metamorphosis, activity of the enzyme increased. Verkuil (1978) also demonstrated the increase in the activity of the enzyme with increasing age of the pupa of *Calliphora erythrocephala.* The rate of metamorphosis obviously depends on the rate of autolytic degradation of tissues which is determined by the activity of acid phosphatase. The tail of *R. tigrina* tadpole undergoes maximum autolysis during metamorphosis. Correspondingly, the tail phosphatase activity has been found to be higher than the other two. Acid phosphatase has been reported to exist in multiple forms in the gut of *P. leavis* (Saleem and Alikhan 1974) and egg of *M. lineatus* (Muthukrishnan and Senthamizhselvan 1985). Certain key enzymes occur in multiple forms, so that inhibition of one form of the enzyme by any factor does not seriously affect the physiological process as it is taken over by the other form(s) of the enzyme (Markert 1975). Metamorphosis, an important event in the life history of *R. tigrina* is controlled by multiple forms of acid phosphatase. It is possible that each fraction of the enzyme has a specific period of peak activity.

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Laboratory evaluation of bait base for the control of sand coloured rat, *Rattus gleadowi*

MOHD IDRIS, A P JAIN and B K SONI
Coordinating and Monitoring Centre for Rodent Research and Training, Central Arid Zone Research Institute, Jodhpur 342 003, India

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Abstract. Since *Rattus gleadowi* replaces *Meriones hurrianae* and *Tatera indica* after a control operation, studies were planned on the bait preferences of this minor pest species. Results tend to reveal that bajra (*Pennisetum typhoides*) whole grain, bajra flour and wheat grain + 2% arachis oil may be used as effective poison carriers for this sand coloured rat. Additives like sugar and salt did not improve the palatability of the candidate bait material. Average energy requirement of experimental rodents ranges from 70·9 Kj/100 g body wt/24 hr in multiple choice tests to 72·6 Kj/100 g body wt/24 hr in single choice tests.

Keywords. Bait preference; *Rattus gleadowi.*

1. Introduction

Introduction of high yielding varieties and multiple cropping patterns have led to general increase in pest-complex in an agro-ecosystem. Besides this, replacement of major rodent pest (Prakash 1975) by the sympatric rodents in an ecosystem, particularly after a control operation (Brown 1971; Joule and Jameson 1972), creates problem to pest control operators who are unaware of rebuild of population of codominant and less dominant rodent pests. In the Indian arid zone *Meriones hurrianae* and *Tatera indica* are major rodent pests whereas, *Rattus gleadowi* represents only 0·6% of the rodent population (Prakash *et al* 1971). This sympatric species replaced dominant gerbils after a control operation and represented 70% of the rodent population (Mathur R P, unpublished results). To manage this upsurge in the population of *R. gleadowi*, studies were carried out on the bait preferences of this sand coloured rat.

2. Materials and methods

The experimental animals were captured from Ber, *Zyzyphus nummularia* fields at CAZRI Farm, Jodhpur. *R. gleadowi* were acclimatized to laboratory conditions for 3 weeks. Each trial was conducted on individually caged 10 adult (5 ♂♂ and 5 ♀♀) animals and the procedures followed are Jain *et al* (1974) and Prakash *et al* (1975).

The average body weight of test animals was 25·2 g.

3. Results and discussion

3.1 *Texture*

R. gleadowi has preferred whole grains to cracked grains and flours. This indicates that these rats are grainivore rodents and therefore, they have rejected the same food grain in other forms (i.e. flour and cracked form) as they had not previous experience of feeding on them in the field (Barnett 1966; Jain and Prakash 1983). When only flours were provided, they have preferred bajra flour and wheat flour to flours of other grains (table 1). Probably taste has played some role in selection of the baits. However, this is a tiny rodent and therefore, it has discarded large sized grains of cowpea being difficult to handle, as compared to bajra and wheat grains. Further, urad has been rated lowest in preference, which may be due to (i) its hardness, (ii) less familiarity since it is in the desert tract and (iii) its high protein content which requires more water for its metabolism.

3.2 *Selection of bait*

These rats have been selecting bajra and wheat constantly at first and second positions, respectively (tables 1–5). In the final test also when best baits from each test were exposed together, these rats clearly made a choice in the following order: (i) bajra grain, (ii) bajra flour and (iii) wheat grain + 2% arachis oil. Hence these items can be used as baits for poisoning *R. gleadowi* populations. These observations also suggest that

Table 1. Consumption of food grains (g/100 g body wt; Mean ± SE) by *R. gleadowi.*

Baits	Whole grains	Kj	Cracked grains	Kj	Flours	Kj	Roasted grains	Kj
Bajra	5·63 ± 0·81	84·68	5·16 ± 0·58	77·64	5·96 ± 0·78	89·68	4·10 ± 0·40	61·59
Chana	2·51 ± 0·69	35·67	1·96 ± 0·41	27·85	2·51 ± 0·46	35·67	2·80 ± 0·52	39·19
Cowpea	1·78 ± 0·56	24·03	1·83 ± 0·34	24·70	2·23 ± 0·63	30·10	1·88 ± 0·53	24·30
Jowar	4·92 ± 0·78	71·97	2·63 ± 0·52	38·47	2·50 ± 0·37	36·57	1·94 ± 0·36	28·38
Maize	2·15 ± 0·46	31·45	3·26 ± 0·72	47·69	2·50 ± 0·63	36·57	2·79 ± 0·53	40·79
Moong	3·31 ± 0·67	45·65	3·55 ± 0·66	48·96	4·90 ± 0·53	67·59	2·09 ± 0·48	28·80
Moth	2·63 ± 0·61	36·31	1·83 ± 0·45	25·24	2·62 ± 0·52	36·11	2·03 ± 0·47	27·96
Til	2·56 ± 0·51	59·90	2·04 ± 0·61	47·73	3·21 ± 0·43	75·11	1·94 ± 0·50	45·39
Urad	1·92 ± 0·40	28·34	1·05 ± 0·37	15·36	1·02 ± 0·29	14·92	2·44 ± 0·45	35·69
Wheat	5·56 ± 0·69	81·34	4·85 ± 0·81	70·95	4·01 ± 0·62	58·64	1·76 ± 0·45	25·74
Av. Kj/100 g body wt/24 hr		49·90		42·45		48·09		35·78

Table 2. Consumption (g/100 g body wt; Mean ± SE) of different forms of food grains by *R. gleadowi.*

	Whole grain v/s cracked grains				Whole grains v/s flour			
Food items	Whole	Kj	Cracked	Kj	Whole	Kj	Flour	Kj
Bajra	4·34 ± 0·79	65·27	2·35 ± 0·58	35·34	6·81 ± 0·85	102·42	3·83 ± 0·67	57·60
Jowar	2·86 ± 0·42	41·84	2·28 ± 0·46	33·35	3·41 ± 0·77	49·88	4·04 ± 0·84	59·10
Moong	2·57 ± 0·40	35·44	2·15 ± 0·48	29·64	2·13 ± 0·71	29·37	3·26 ± 0·89	44·95
Moth	2·27 ± 0·52	31·30	2·33 ± 0·57	32·13	3·54 ± 0·60	45·81	2·86 ± 0·87	39·43
Wheat	3·00 ± 0·56	44·32	2·41 ± 0·46	35·26	5·09 ± 0·96	74·46	1·01 ± 0·44	14·77
Av. Kj/100 g body wt/24 hr		43·63		33·14		60·98		21·57

Table 3. Food consumption (g/100 g body wt; Mean ± SE) with oils by *R. gleadowi*.

	Whole grains + 2%				Flours + 10%			
Food items	Arachis oil	Kj	Sesame oil	Kj	Arachis oil	Kj	Sesame oil	Kj
Bajra	3·31 ± 0·60	49·78	3·23 ± 0·69	48·57	3·35 ± 0·49	50·38	2·62 ± 0·61	39·40
Jowar	2·14 ± 0·19	31·50	2·27 ± 0·53	33·21	2·17 ± 0·55	50·79	2·32 ± 0·54	54·30
Moong	3·15 ± 0·24	43·43	2·29 ± 0·44	31·57	3·33 ± 0·70	48·71	2·05 ± 0·54	29·99
Moth	2·03 ± 0·43	27·99	2·97 ± 0·45	40·95	2·80 ± 0·57	38·61	2·12 ± 0·52	29·23
Av. Kj/100 g body wt/24 hr		42·22		36·85		45·13		36·02

Table 4. Food consumption (g/100 g body wt; Mean ± SE) with salt and sugar by *R. gleadowi*.

	Whole grains + 2% Arachis oil				Flours + 10% Arachis oil + 1%			
Food items	Salt	Kj	Sugar	Kj	Salt	Kj	Sugar	Kj
Bajra	2·46 ± 0·45	37·02	3·85 ± 0·52	57·90	5·21 ± 0·38	78·35	2·26 ± 0·54	33·99
Jowar	3·09 ± 0·52	45·20	3·23 ± 0·48	47·30	1·67 ± 0·59	24·43	1·00 ± 0·39	29·26
Moong	2·81 ± 0·41	30·74	2·28 ± 0·55	31·44	4·63 ± 0·36	63·64	2·58 ± 0·50	35·57
Moth	2·05 ± 0·43	28·26	2·65 ± 0·27	20·26	—	—	—	—
Til	—	—	—	—	3·16 ± 0·54	73·96	0·95 ± 0·33	22·23
Wheat	1·22 + 0·28	17·85	2·15 + 0·38	31·89	4·73 + 0·54	69·19	1·40 + 0·33	20·48
Av. Kj/100 g body wt/24 hr		33·41		37·75		61·95		28·30

Table 5. Relative consumption of best ranking food items from each test.

Baits (best from each test)	Average daily intake (g) (Mean ± SE)	Rank	Kj (100 g body wt/day)
Bajra, whole grains	6·11 + 0·32	1	91·91
Bajra, cracked	2·24 ± 0·32	10	33·69
Bajra, flour	4·63 + 0·55	2	69·63
Bajra, roasted	2·82 ± 0·44	7	42·42
Bajra, flour + 10% arachis oil	3·80 ± 0·60	3	56·97
Bajra, flour + 10% arachis oil + 1% salt	2·72 + 0·46	8	40·92
Jowar, flour	3·55 ± 0·48	5	51·91
Wheat, whole grains	2·41 ± 0·34	9	35·23
Wheat, whole + 2% arachis oil	3·89 ± 0·64	4	56·88
Wheat, whole + 2% arachis oil + 1% salt	2·91 ± 0·41	6	42·55

R. gleadowi is a selective feeder and is a pest of bajra and wheat. In nature, it is associated with *Tatera indica* and *Meriones hurrianae* (Prakash *et al* 1971) which are recognised pests of wheat and bajra (Advani *et al* 1982).

Desert rodents depend mostly on preformed water in food and metabolic water (Maloiy 1979). Ingestion of high protein food may adversely affect their water budget. Therefore, least preference for high protein food (urad 24% and cowpea 24·1% protein) as compared to bajra (11·6%) and wheat (11·8%) (Gopalan *et al* 1977), is an expected adaptation to xeric condition (tables 1–5).

3.3 *Food preference in no-choice and choice tests*

There was no significant difference in the average daily intake of same food when provided singly or in multiple choice (table 6). This leads to selection of superior food and therefore, *R. gleadowi* preferred bajra 9, 3 and 2 times at first, second and third positions, respectively whereas, wheat was liked 2, 5 and 2 times on similar positions (tables 1–6). Interestingly bengal gram (once) and moong (twice) attained second position whereas, jowar (thrice), moong (twice), maize and moth (once) also appeared at 3rd position in preference tests (tables 1–6). This indicated dietary variability and it is expected in desert ecosystem for survival.

3.4 *Role of additives*

Addition of arachis oil as well as sesame oil retarded the average daily intake of most of the whole grains to the extent that the difference attained statistical level of significance in case of bajra and jowar ($P<0{\cdot}05$, table 3). Similar case with flours but the difference in consumption attained statistical level of significance only in case of bajra ($P<0{\cdot}05$, table 3). It is also observed that this difference is elaborated in cases of moong and wheat with addition of sesame oil only ($P<0{\cdot}05$) and not with addition of arachis oil. Further, addition of sugar and salt also reduced average daily intake of all the food grains tested (table 4), but the difference attained significant level in case of bajra with salt ($P<0{\cdot}05$) only. In case of flours though reduction in average daily intake is observed (table 4) but significant difference is noticed when sugar is added to bajra, moong, til and wheat ($P<0{\cdot}05$). It is therefore, pertinent to conclude that role of additive may be visualised in perspective of this foregoing analysis. Since addition of oils and salt and sugar have adversely affected the average daily intake, their use in any management strategy should be done with utmost care. Under field conditions, therefore, the role of oil is not more than a binder of poison to the grains. More quantities of oil than the desired ones, may have deterrent effects on the consumption of baits (Fitzwater and Prakash 1978; Jain and Prakash 1983). Moreover, use of sugar and salt may be avoided for all practical purposes.

Table 6. Calorific value and palatability indices of various food items in choice and no-choice tests.

Baits without additives	Average daily intake (g)/100 g body wt Multiple food choice (Mean ± SE)	Kj	Average daily intake g/100 (g) body wt single food choice (Mean ± SE)	Kj	Palatability index: Values between multiple and single food choices
Bajra	5·63 ± 0·81	84·68	5·87 ± 0·29	87·90	0·28 NS
Jowar	4·92 ± 0·78	71·97	5·06 ± 0·29	74·02	0·16 NS
Moong	3·31 ± 0·67	45·64	3·90 ± 0·10	53·79	0·88 NS
Wheat	5·56 ± 0·69	81·34	5·17 ± 0·19	75·61	0·78 NS
Av. Kj/100 g body wt. 24/hr	4·85	70·92	5·00	72·83	

NS, Not significant.

3.5 *Energy budget*

The energy requirement of these rats ranged from 53·8–87·9 Kj (av. 72·8 Kj)/100 g body wt/24 hr in single food choice to 45·6–84·6 Kj (av. 70·9 Kj)/100 g body wt/24 hr in multiple choice tests (table 6). The low energy budget of this rodent as compared to other desert rodents (*T. indica*—Jain and Prakash 1983; *Meriones hurrianae*—Prakash and Kumbkarni 1962; *Rattus c. cutchicus*—Jain *et al* 1975; *Gerbillus gleadowi*—Prakash *et al* 1975; *Rattus meltada*—Jain *et al* 1974), probably indicated that its range of movement is small and it prepares very simple burrows (Advani and Jain 1983) which does not warrant high energy budget.

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On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae)

R J LAZARUS and P SITA RAMI REDDY
Department of Zoology, Madras Christian College (Autonomous), Tambaram, Madras 600 059, India

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Abstract. The length-weight relationship was computed independently for males and females of *Macrognathus aculeatus*. The analysis of covariance revealed no significant difference ($p = 0.05$) between the length-weight relationship of males and females. Hence, a common logarithmic equation ($\log W = -5.6934 + 3.1234 \log L$) was fitted for the species. The regression coefficient of logarithm of weight on logarithm of length was found to depart significantly ($p = 0.05$) from the cubic value.

Keywords. Length-weight relationship; *Macrognathus aculeatus;* logarithmic equation; regression coefficient; cubic value.

1. Introduction

The mathematical relationship between length and weight of fishes and its vital importance in fishery is well known to fishery biologists. The study of length-weight relationship provides information to measure variation from the expected weight or length of individual fish or group of fish as indicative of fatness, general well-being or gonad development (Le Cren 1951). This is of primary importance in computing the yield equations (Ricker 1958), in estimating the number of fish landed and in comparing populations in time and space (Chanchal *et al* 1978). Of the two measurements, i.e., length and weight, the former is more easily measurable and can be converted into weight, in which the catch is invariably expressed (Bal and Rao 1984).

Macrognathus aculeatus (Bloch) inhabits freshwater streams, ponds and tanks. They attain upto 15 inches in length and are excellent as food (Day 1878). As they are often found in pieces of water in which mud abounds, they are popularly known as the 'ditch eels' (Hora 1935). They are also known as the 'lesser spiny eels' (Munro 1955).

Though *M. aculeatus* is found to be a commercially important species, there is no information on its biology. Therefore, an attempt has been made to study its length-weight relationship, with the two-fold aim of determining (i) whether the length-weight relationship differs between the sexes, and (ii) whether the coefficient of regression of logarithm of weight on logarithm of length departs significantly from the cubic value.

2. Materials and methods

The length-weight relationship was based on the data obtained from a total of 110 fishes, comprising 25 males (153–225 mm in total length) and 85 females (170–317 mm in total length), collected in fresh condition from the local fish market at Tambaram during May 1984–June 1985. The total length of the fish was measured from the tip of the snout to the tip of the tail and expressed in mm. The body weight was recorded to the nearest 0·001 g. As there was no sexual dimorphism, the fishes were dissected and sex was determined.

The length-weight relationship was calculated by employing Le Cren's (1951) hypothetical formula: $W = aL^n$ and its logarithmic form: $\log W = \log a + n \log L$. The regression of logarithm of weight on logarithm of length of males and females was tested by analysis of covariance (Sokal and Rohlf 1969). The *t*-test (Sokal and Rohlf 1969) was employed to test whether the regression coefficient departs significantly from the cubic value.

3. Results

The observed values of length and weight of *M. aculeatus* were plotted (figure 1). The parabolic equations obtained were:

Males: $W = 0{\cdot}00000152229\ L^{3{\cdot}1663}$
Females: $W = 0{\cdot}00000466015\ L^{2{\cdot}9712}$

Similarly, the logarithmic values of observed length and weight were plotted (figure 2). The regression lines fitted to the data indicate straight line relationship

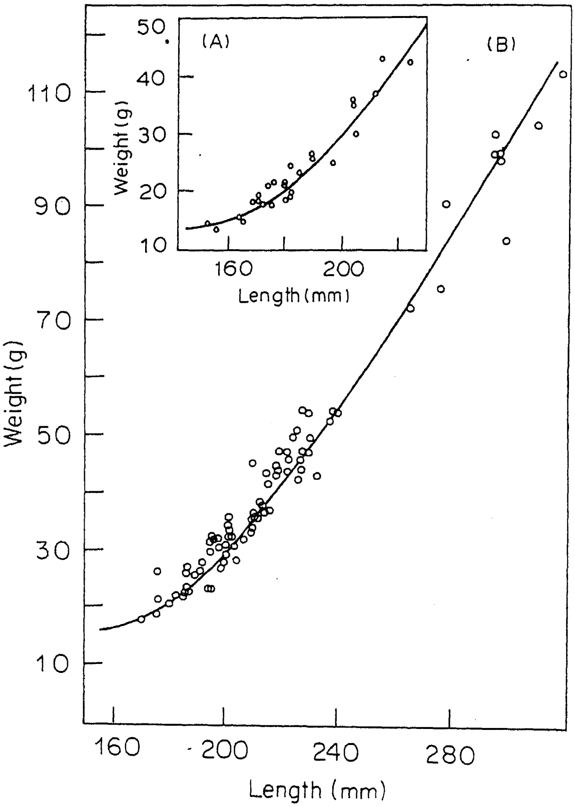

Figure 1. Observed values of length and weight of *M. aculeatus*. **(A)** Males; **(B)** Females.

between the two variables. The logarithmic equations obtained were:

Males: $\log W = -5{\cdot}8175 + 3{\cdot}1663 \log L$
Females: $\log W = -5{\cdot}3316 + 2{\cdot}9712 \log L$

Analysis of covariance revealed no significant ($p = 0{\cdot}05$) difference between the sexes (tables 1–3). This has justified the pooling of data of the two sexes to obtain the following common equations for the species:

Parabolic equation: $W = 0{\cdot}00000202581\ L^{3{\cdot}1234}$
Logarithmic equation: $\log W = -5{\cdot}6934 + 3{\cdot}1234 \log L$.

The t-test revealed that the value of regression coefficient (3·1234) obtained for this species departed significantly from the cubic value (3) at 5% level with 108 df.

4. Discussion

For fishes, the relationship between length and weight could be expressed by the hypothetical cube law: $W = c\ L^3$, where 'W' represents weight of the fish, 'L' its length and 'c' a constant. Le Cren (1951), however, opined that it is better to fit a general

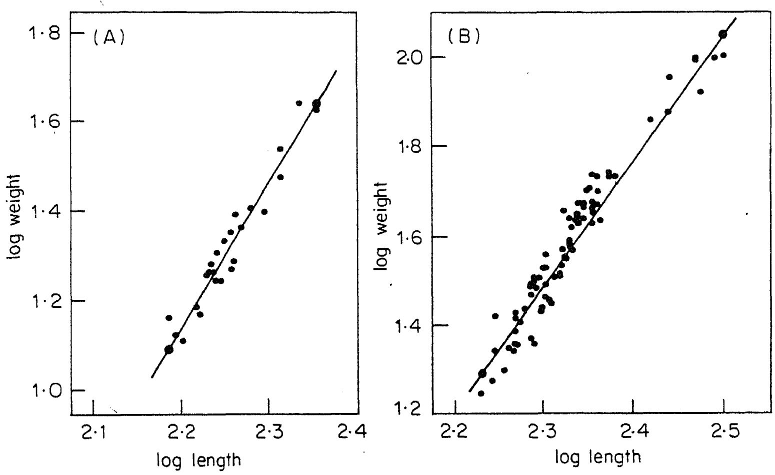

Figure 2. Logarithmic values of observed length and weight of *M. aculeatus*. (**A**) Males; (**B**) Females.

Table 1. Statistics of the length-weight relationship of males and females of *M. aculeatus*.

Sex	N	SX	SY	SX²	SY²	SXY
Male	25	56·4296	33·2360	127·4114	44·6108	75·1444
Female	85	197·6420	134·0447	459·8712	214·1631	312·6138

SX², SY², SXY, Sum of squares and products; SX, SY, Sum of logarithmic values of length and weight, respectively; N, Number of samples.

Table 2. Regression data of length-weight relationship of males and females of *M. aculeatus*.

		Sum of squares and products				Errors of estimate	
Sex	df	X^2	Y^2	XY	b	df	SS
Male	24	0·0394	0·4255	0·1246	3·1663	23	0·0309
Female	84	0·3183	2·7751	0·9330	2·9712	83	0·0398
	108	0·3577	3·2006	1·0576		106	0·0707

df, Degree of freedom; b, regression coefficient; SS, sum of squares.

Table 3. Test of significance.

Source of variation	df	SS	MS	Observed F	F=0·05
Deviation from individual regression between sexes	106	0·0707	0·000666037		
Deviation between regressions	1	0·0016	0·0016	2·4024	3·92 Not significant
Deviation from total regression	107	0·0722			

parabolic equation of the form $W = a\ L^n$, where 'a' is a constant equivalent to 'c' and 'n' is a constant to be determined empirically, i.e., from the data.

Presently, it has been found that the males increased in weight by an exponent (regression coefficient) of 3·1663 and females by 2·9712. Though the value of 'n' was slightly higher in males than in females, this difference in exponent values was not significant ($p = 0{\cdot}05$). However, similar higher values for males than females have been reported in the case of *Sardinella gibbosa* (Sekharan 1968), *Labeo calbasu* (Pathak 1975) and *Anabas testudineus* (Chanchal *et al* 1978).

For an ideal fish that maintains constant shape, the value of 'n' will be 3 (Allen 1938), i.e., the weight will be proportional to the cube of length if the fish does not change form or density as it grows. Changes in morphology with advancing age may, however, cause the value of 'n' to depart significantly from 3, so much so that the exponent 'n' in the parabolic equation usually lies between 2·5 and 4·0 (Hile 1936; Martin 1949). Beverton and Holt (1957) stated that important deviations from isometric growth in adult fishes are rare. According to Ricker (1958), a good number of fishes approach ideal condition, though deviations from the hypothetical cubic value are not uncommon. A number of factors, viz gonad development, season, physiological condition of the fish at the time of collection and the nutritive condition of the water from which the fish were collected (Chanchal *et al* 1978) or food and feeding habits of the fish (Soni and Kathal 1979) may influence the value of 'n'. Presently the value of regression coefficient obtained for *M. aculeatus* (3·1234), when subjected to the t-test, revealed that it did depart significantly ($p = 0{\cdot}05$) from the cubic value (3). Deviations from the isometric value of 3 have been reported in many species of fishes (Jhingran 1962; Antony Raja 1967; Sinha 1972; Rangaswamy 1976; Rita Kumari and Balakrishnan Nair 1978; Nasar and Biswas 1982; Lazarus and Reddy 1986).

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Feeding ecology of the large grey babbler *Turdoides malcolmi*

H S TOOR and MANJIT S SAINI
Department of Zoology, Punjab Agricultural University, Ludhiana 141 004, India

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Abstract. Studies on the feeding ecology of large grey babbler *Turdoides malcolmi* (Sykes) were carried out during August 1981 to July 1982 at Ludhiana. Observations on feeding behaviour and feeding associations with other bird species were recorded throughout the season. Gut contents of 125 adults and 20 nestlings revealed that this species is mainly insectivorous and annual percentage of animal matter in the diet was found to be 62 and 93 respectively.

Keywords. *Turdoides malcolmi*; feeding ecology; economic importance; large grey babbler; birds.

1. Introduction

Detailed analysis of food and feeding behaviour of different bird species of a region is the first and foremost requirement to assess their economic status and to initiate work on bird management in that region. Unfortunately, only limited information is available on the feeding ecology of Indian birds. As a sequel, the economic status of many bird species remains yet to be defined accurately. Large grey babbler *Turdoides malcolmi* (Sykes) is one of the common bird species of India. Ali and Ripley (1971) have stated this babbler to be an omnivorous species but detailed information on its feeding ecology is lacking. Therefore, the present studies were undertaken with an aim to determine its economic status in the agroecosystem of Punjab.

2. Material and methods

Studies were carried out during August 1981 to July 1982 in the field area of Punjab Agricultural University, Ludhiana (30°56′N, 75°52′E and *c.* 247 m above the mean sea level) and its surrounding areas. This area is intensively farmed, mainly having two crop seasons—*kharif* (June–August to September–December) and *rabi* (October–November to April–May). *Kharif* crops include rice, cotton, maize, pearl millet, groundnut, sugarcane, vegetables (cucurbits, okra, brinjal, tomato, chillies etc.) and pulses (*arhar, urd*). The *rabi* crops are wheat, *toria, raya*, sugarcane, pulses (lentil and gram), and vegetables (cauliflower, cabbage, knol khol, radish, turnip, carrot). Climate of the study area is a semi-arid monsoon type. Generally, 4 seasons are experienced in a year: summer or pre-monsoon (April–June), southwest monsoon (July–September), post-monsoon (October–November) and winter (December–March). The babblers were observed through 7 × 50 field binoculars periodically at different times of the day in different regions of the study area to record observations on their feeding behaviour, feeding sessions, size of flocks and feeding associations with other birds. Gut contents of 125 adults were studied gravimetrically. For this purpose babblers were shot periodically with a 0·22 air gun; either early in the morning or late in the evening when they were feeding actively. Whenever it became too late to analyse the gut contents of babblers collected in the evening, their gizzards (there being no crop) were dissected out, labelled with price tags and put in 10% formaldehyde solution. On the subsequent day, gizzards were deformalised by keeping in water for about 15 min and then dissected out for analysing contents. After weighings of gut contents, plant matter was preserved dry, while animal matter was preserved in 70% alcohol for further identification. During the 1982 breeding season (May–September), 20 nestlings of the babbler were collected in the morning hours and their gut contents were analysed gravimetrically.

3. Results and discussion

3.1 *Feeding behaviour*

Large grey babblers are resident in the study area. They inhabit low vegetation and prefer gardens, orchards etc. Flock size varied in these birds from 2–12 members. The birds usually fed near the bushes and trees, hopping around here and there in low grass, picking up insects and plant matter. The babblers were also seen taking bites from the leaves of shisham *Dalbergia sissoo, 'beri' Zizyphus jujuba,* and mulberry *Morus* spp. trees and capture ants and other insects from tree branches with swift movements of their head. From August–October, both adult and immature babblers were observed to peel off the maize cobs and feed on the grains at different stages of maturity. They entered pearl millet fields only for insects. In November, when rice is harvested babblers were seen feeding on the fallen rice grains from the ground. During whole of the winter season the birds consumed large quantities of plant matter. At the sowing of wheat, they picked up superficially left seeds and during April–May fed on the wheat grains from the ground where they fall during crop harvest. In the monsoon season, babblers were observed devouring winged termites emerging from termite mounds. They often caught them in mid-air by taking swift take off in the black drongo *Dicrurus adsimilis* manner.

3.2 *Feeding associations*

Large grey babblers were observed foraging on the ground and nearby bushes frequently in the association of common myna *Acridotheres tristis*, pied myna *Sturnus contra*, hoopoe *Upupa epops* and Indian robin *Saxicoloides fulicata.* On 3 occasions these were seen feeding near grey partridge *Francolinus pondicerianus.* In the orchards and gardens, babblers were seen searching for food in association with the crow pheasant *Centropus sinensis*, hoopoe and pied crested cuckoo *Clamator jacobinus.* Sometimes, they were observed capturing ants from tree branches in association with the redvented bulbul *Pycnonotus cafer*, rufousbacked shrike *Lanius schach*, common myna and goldenbacked woodpecker *Dinopium benghalense.* In paddy and wheat fields the babblers fed near house sparrow *Passer domesticus* and weaverbirds *Ploceus* spp.

3.3 *Food of the adults*

The data on relative percentage (dry weight) of various items in the diet of large grey babblers during different months are presented in table 1. Animal matter constituted 62% of the total gut contents, as against 36·92% of plant matter and 1·07% grit. Among animal matter, insects (43·73%) formed major portion of diet, followed by lizards (7·39%). Small portions of snail shells (0·43%) and Arachnids (0·12%) were also recorded. The insect matter was represented by body parts of ants (Hymenoptera), beetles (Coleoptera), grasshoppers (Orthoptera), caterpillars (Lepidoptera), flies (Diptera), dragon flies (Odonata) and termites (Isoptera). Order Lepidoptera was the most abundant among insects which constituted 15·75% of total food and was recorded in 33 guts. At second place was Orthoptera (10·34%), which in turn was followed by Hymenoptera, Coleoptera, Isoptera, Odonata and Diptera.

The major portion (24·92%) of plant matter in the gut contents could not be identified. Wheat grains (5·55% of total contents) were found in 21 guts and were the most common food item in the identified portion of the plant matter. Maize grains ranked second and rice was found in only small quantities. Leafy material constituted 1·58% of total food and was recorded in only 3 guts. Weed seeds of wild '*senji*' *Melilotus indica, 'hulhul' Cleome viscosa* and *'kaon makki' Commelina benghalensis* collectively formed only 0·64% of total food.

During pre-monsoon and monsoon season (breeding season of babbler) large grey babblers consumed large quantities of animal matter. The bulk of this was constituted by insects of which grasshoppers, caterpillars, beetles and ants were taken in significant quantities. Increased insect consumption by adult babblers during their breeding season was probably owing to the increased availability of insects as well as the limited available foraging time due to high energy demands of the breeding activity. Hintz and Dyer (1970) also extended the same view while describing the food of red-winged black birds.

The frequency of occurrence of body parts of beetles and ants in the gut contents of babblers was more than any other insect group. It may not be true to consider that these are the preferred food items because these hard bodied insects are digested comparatively slowly and thus usually recovered in more guts. Coleopteran parts have been reported to remain for longer time in the gizzards of starling *Sturnus vulgaris* and their importance gets exaggerated (Kalmbach and Gabrielson 1921). Moeed (1980) has also supported this view.

Presence of snail shells (April–July) in the diet of babblers can be attributed to the increased calcium requirement for egg laying. Plant matter was represented by wheat, maize and leafy material in these seasons. Since grit was found in small quantities, hard body parts of insects (e.g., mandibles) may be performing the function of grit in the gizzards. Bird and Smith (1964) and Mott *et al* (1972) have also observed that in red-winged blackbird the least amount of grit was picked up when insects formed a large portion of the diet.

During winter season, percentage of animal matter consumed was less owing to the less availability of insects and spiders. However, the percentage of Lepidoptera in gut contents increased during these months showing that these babblers feed upon the hibernating larvae. Main identified portion of plant matter was represented by wheat and rice grains while weed seeds were found in traces.

3.4 *Food of the young*

Large bulk (93%) of gut contents of the young consisted of animal matter while plant matter accounted for 6·94% only (table 2). Presence of plastic pieces in the food

Table 1. Relative percentage (dry weight) of different food items in the guts of adult large grey babbler.

Food items	Aug $n=11$	Sept $n=12$	Oct $n=12$	Nov $n=9$	Dec $n=11$	Jan $n=10$	Feb $n=9$	Mar $n=10$	Apr $n=11$	May $n=9$	Jun $n=10$	Jul $n=11$	Annual average $n=125$
Animal matter													
Phylum chordata Class Reptilia (lizards)	—	50·70 (2)	—	—	—	—	—	—	—	—	—	—	7·39 (2)
Phylum Mollusca (snails)	—	—	—	—	—	—	—	—	2·43 (3)	5·71 (3)	0·12 (1)	0·55 (1)	0·43 (8)
Phylum Arthropoda Class Insecta													
Coleoptera	5·48 (2)	3·23 (6)	7·39 (3)	0·32 (1)	0·35 (1)	—	—	15·63 (9)	7·91 (6)	12·78 (6)	10·04 (8)	7·90 (5)	5·36 (47)
Orthoptera	28·36 (4)	8·81 (3)	26·47 (3)	—	—	—	—	—	17·17 (3)	8·67 (2)	8·92 (3)	22·37 (4)	10·34 (22)
Lepidoptera	4·74 (1)	1·02 (1)	2·24 (1)	23·47 (3)	32·27 (3)	39·47 (4)	23·38 (2)	4·63 (3)	14·64 (5)	28·19 (4)	18·60 (3)	9·59 (3)	15·75 (33)
Diptera	—	—	—	—	—	—	—	1·54 (2)	0·75 (2)	1·37 (3)	0·44 (1)	0·29 (1)	0·26 (9)
Odonata	—	—	—	—	—	—	—	—	—	5·82 (2)	1·32 (1)	—	0·36 (3)
Hymenoptera	26·0 (6)	13·07 (7)	12·83 (5)	2·03 (2)	0·71 (2)	12·17 (6)	10·42 (3)	14·73 (8)	8·47 (6)	10·62 (6)	3·09 (4)	5·95 (7)	11·20 (62)
Isoptera	—	—	—	—	—	—	—	—	—	4·51 (5)	1·43 (3)	2·05 (3)	0·46 (11)
Total insects	64·58	26·13	48·93	25·82	33·33	51·64	33·80	36·53	48·94	71·96	43·84	48·15	43·73
Class Archnidae (spiders)	0·17 (1)	*T* (1)	—	—	—	—	—	0·75 (2)	0·27 (1)	0·51 (1)	—	—	0·12 (6)

Animal matter (unidentified)	16·03 (5)	2·06 (1)	23·33 (3)	5·83 (1)	11·93 (3)	2·07 (1)	15·56 (2)	1·99 (1)	11·83 (3)	4·74 (1)	8·89 (3)	19·15 (4)	10·33 (28)
Total animal matter	80·78	78·79	72·26	31·65	45·26	53·71	49·36	39·27	63·49	82·92	52·85	67·85	62·00
Plant matter													
Maize	9·13 (2)	12·75 (4)	3·46 (1)	—	—	—	—	—	—	—	—	—	3·41 (7)
Wheat	—	—	—	10·40 (1)	0·81 (1)	0·67 (1)	7·63 (2)	17·52 (2)	12·22 (6)	1·54 (1)	21·93 (4)	2·96 (3)	5·55 (21)
Rice	—	—	—	0·64 (1)	1·45 (2)	—	—	—	—	—	—	—	0·14 (3)
Weed seeds	0·98 (1)	0·53 (2)	2·84 (2)	—	1·98 (1)	—	—	—	—	1·26 (1)	—	—	0·64 (7)
Crushed seeds (unidentified)	—	—	—	—	—	9·69 (2)	9·53 (1)	—	—	—	—	—	1·58 (3)
Leafy material	—	—	—	—	—	—	—	3·96 (3)	3·12 (1)	—	1·40 (1)	1·69 (3)	0·68 (8)
Plant matter (unidentified)	9·10 (5)	7·76 (8)	21·44 (9)	57·31 (9)	50·49 (6)	35·92 (9)	33·48 (7)	38·43 (5)	21·16 (3)	7·71 (3)	19·10 (5)	22·99 (6)	24·92 (74)
Total plant matter	19·21	21·04	27·74	68·35	54·73	46·28	50·64	59·91	36·50	10·51	42·43	27·64	36·92
Grit	—	—	—	—	—	—	0·79 (1)	—	6·56 (5)	4·69 (4)	4·49 (2)	—	1·07 (12)

n, Number of guts analysed; *T*, traces (<0·1%).

Figures in parentheses indicate frequency of occurrence of food items.

Table 2. Food of the young of large grey babbler *T. malcolmi*.

Food item	Mean percentage (wet weight) of food items in the guts		
	Nestlings $n=11$	Fledgelings $n=9$	Nestlings and fledgelings $n=20$
Animal matter			
Molluscs (snails)	1·71 (8)	0·92 (4)	1·25 (12)
Insects			
Orthoptera (grasshoppers)	32·15 (7)	1·32 (1)	14·19 (8)
Coleoptera (beetles)	5·33 (8)	1·43 (5)	3·06 (13)
Lepidoptera (caterpillars)	46·86 (7)	83·19 (9)	68·03 (16)
Hymenoptera (ants)	1·44 (4)	—	0·6 (4)
Total insects	85·78	85·94	85·88
Arachnida (spiders)	0·82 (1)	—	0·34 (1)
Unidentified animal matter	3·49 (1)	6·99 (3)	5·53 (4)
Total animal matter	91·80	93·85	93·00
Plant matter	8·06 (9)	6·15 (5)	6·94 (14)
Plastic pieces	0·15 (1)	—	*T* (1)

n, Number of guts analysed; *T*, traces (<0·1%).
Figures in parentheses indicate frequency of occurrence of particular food items.

consumed was unusual. Major part (85·88%) of the animal matter was constituted by insects. Molluscs formed 1·25% and Arachnids only 0·34%. Among insects, caterpillars were the main food item in the consumed food while grasshoppers, beetles and ants were the other preferred insects in the descending order. Most of the young birds are often given soft bodied invertebrates and that too of large size (Coleman 1977). Since the growth in young is very fast they need large quantities of proteinous food and perhaps because of this, it is not worthwhile for adults to make flights to the nest with small food items when they can find larger ones in the same area. Our observations also suggest the same because grasshoppers and caterpillars, which were fed in large quantities to the young ones, are comparatively large animals. The diet of nestlings and fledgelings was apparently the same (table 2).

3.5 *Economic status*

The adult babblers are omnivorous i.e., they feed on a wide variety of food items viz insects, arachnids, molluscs, wheat, maize, rice, weed seeds and leafy material. Among

Table 3. Animals identified from the gut contents of large grey babbler.

Animal	Adults	Young	Remarks
Phylum Chordata			
Class Reptilia			A common lizard; body parts of young lizards recovered from guts
Order Squamata			
Family Agamidae			
Calotes sp.	+	–	
Phylum Mollusca			
Class Gastropoda			Aquatic snail; host of a stomach fluke (*Orthrocoelium streptocoelium*) which causes Paramphistomiasis disease of livestock
Order Basommatophora			
Family Planorbidae			
Bulimus tentaculatus	+	+	
Order Monotocardia			
Family Melaniidae			
Melanoides tuberculatus	+	+	
Phylum Arthropoda			
Class Insecta			Phytophagous weevils; damage stored grains; stem borers and leaf feeders
Order Coleoptera			
Family Curculionidae	+	+	
Sipalus sp.	+	–	
Myllocerus sp.	+	–	
Astycus sp.	+	+	
Episomus sp.	–	+	
Family Chrysomelidae			
Aulacophora faveicollius	+	–	Serious pests of cucurbits
Family Bupenstidae			
Rhizopertha dominica	–	+	Stored grain pest
Family Carabidae	–	+	Mutilated body parts; very destructive to groundnut and sugarcane crops during their immature stages
Family Elateridae	+	+	
Family Cantharidae	–	+	Body parts found; adults damage flowering plants
Order Hemiptera			
Family Pentatomidae	+	–	Mutilated body parts found; members suck sap of plants resulting into their wilting
Order Diptera			
Family Calliphoridae			
Calliphora sp.	+	–	Saprophagous fly
Order Hymenoptera			
Family Formicidae			
Cataglyphus bicolor	+	+	
Componotus sp.	+	–	
Order Orthoptera			
Family Acrididae			
Colemania sp.	+	+	
Acrida sp.	+	+	Polyphagous hopper; feeds on leaves of rice, grass, millets
Aiolopus sp.	–	+	

Table 3. (*Contd.*)

Animal	Adults	Young	Remarks
Class Arachnida			
Order Aranae	+	+	Spiders generally are predators of insect pests and thus useful animals
Family Linyphidae			
Linyphia sp.	+	–	
Family Argiopidae			
Argiope sp.	+	–	
Family Salticidae			
Marpissa ludhianaensis	+	+	
Family Oxypidae			
Oxyopes sp.	+	–	
Family Lycosidae			
Pardosa bhatnagari	–	+	

+, Present; –, absent.

animal matter major portion of their food is formed by insects out of which most ar harmful viz curculionids (pests of stored grains), chrysomelids (pests of cucurbits pentatomids (pests of many plants; they generally suck sap resulting in the wiltin and dying away of plants), bupenstids (mostly stored grain pests), carabids (calle white grubs; very destructive to groundnut and sugarcane crops during immatur stages) and Orthopterans (mostly phytophagous hoppers). The snails consumed b babblers are known to be the vectors of infectious diseases of live stock (table 3).

During the post-monsoon season, babblers feed on maize but the percentage this food item is very less. Wheat and rice grains are taken in small quantities an those too from the ground, where they fall as a result of damage caused by othe birds to the standing crops. Weed seeds are also consumed by the adult babblers bu in very small quantities and, therefore, there is not much significance of thes babblers as dispersing agents of the weeds. The young ones are fed mainly on thos insects which are in no way useful to the humans. The only useful animals fed to th young are spiders but they make only up to 0·61% of the total food.

Our studies, therefore, tend to conclude that the large grey babbler on the whole a useful species to mankind.

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Distribution and zonation of shore crabs in the Pulicat lake

D R JOEL*, P J SANJEEVA RAJ** and R RAGHAVAN*
Estuarine Biological Laboratory, Pulicat Chengleput District, Tamil Nadu, India
Present address: *Fisheries College of Tamil Nadu Agricultural University, Tuticorin 628 008, Tamil Nadu, India
**CRNIEO, Department of Ecology and Aquaculture, No. 1 First Street, Haddows Road, Madras 600 006, India

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Abstract. Twentynine species of crabs encountered along the shores of the Pulicat lake have been surveyed with special reference to their vertical distribution, zonation of discrete patches, seasonal variation in population densities and the extent of their penetration into the lake. Specific association between vertical range and moisture content of the soil, zonation of discrete patches and composition of the substratum and reduction in number or total disappearance of crabs and lowering of salinity caused by monsoonal floods were discussed.

Keywords. Pulicat; shore; crabs; distribution.

1. Introduction

Alcock (1896–1900) contributed comprehensive information on the taxonomy and geographical distribution of most of the Indian crabs. Further understanding of the distribution pattern of the shore crabs was mainly due to the works in the brackish ponds and on the bank of the Malta river of Port Canning (de Man 1908–09; Pearse 1932), in the littoral region of Krusadai Island (Gravely 1927), in the brackish waters around Madras (Panikkar and Aiyar 1937), on the Karwar coast and the neighbouring islands (Patil 1951), in the Mandapam area (Sankarankutty 1965), at the Vellar estuary (Balasubramanyan 1966) and on the shore of Bombay (Balani 1975).

A perusal of the literature reveals that investigations on ecological distribution of crabs have been confined either to the open shores, bays or to the river-mouth estuaries in India. Thus very little attention seems to have been paid to the major brackish water lakes. Therefore the present study was undertaken with a view to determining the factors behind the vertical distribution, zonation of discrete patches, seasonal fluctuations in population densities and penetration of shore crabs in the Pulicat lake on the east coast of India.

2. Materials and methods

The observations and results of monthly surveys were made for the year 1972 and further confirmative but irregular observations continued till 1978, on the shore crabs at 4 stations fixed along the length of the Pulicat lake from south to north (figure 1). The comparative and semiquantitative method of Endean *et al* (1956) was followed to estimate the relative abundance of crabs encountered along the traverse made at each station. The key to denote the abundance for a 50 metre radius

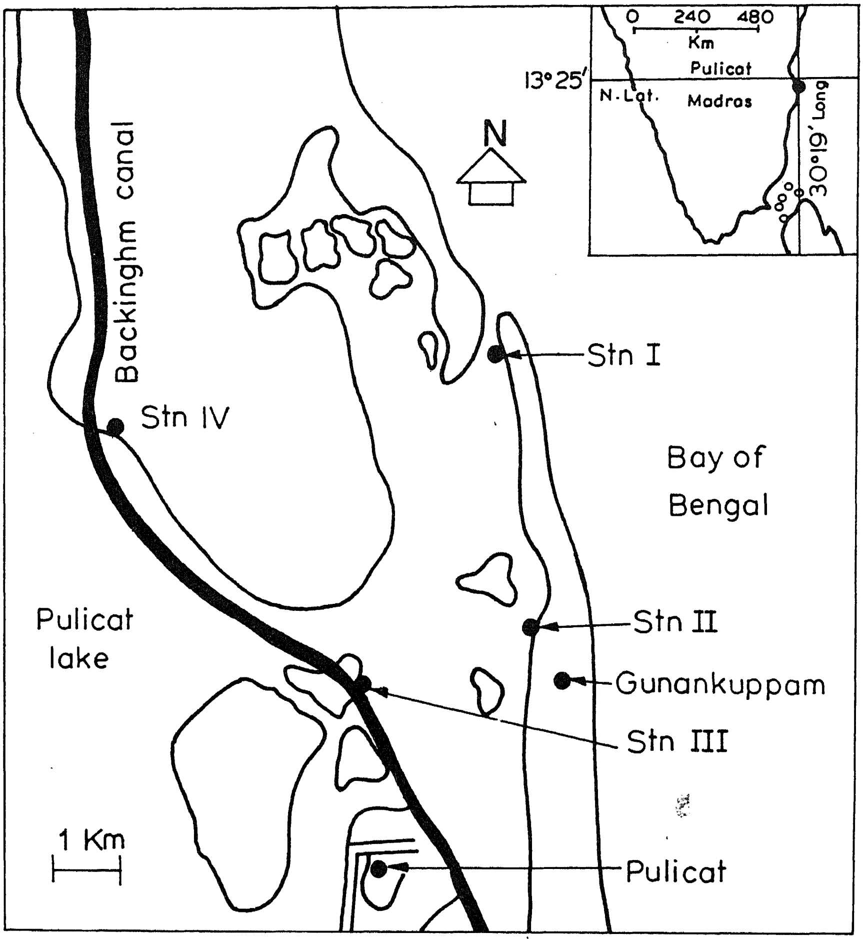

Figure 1. Sampling stations in Pulicat lake.

followed: Dominant and co-dominent, more than 30 individuals; sub-dominent between 25 and 30 crabs; very common, between 20 and 25 crabs; common, less tha 20 but more than 5; present, between 5 and 2; rare, 1.

Month-wide density of each crab species was arrived at by averaging th populations by the corresponding number of the 4 southern stations wherein th respective species was distributed. Apparently if a single species was observed only i one particular station, that population alone was considered as the density. Th fractions were rounded upto the nearest number.

3. Hydrography of the lake

3.1 *Salinity*

During the north east monsoon period (October–December) the salinity wa generally brackish (table 1), but low salinity values (0·5–2·4%) immediately afte

Table 1. Annual variations in temperature and salinity.

Parameters	Non-monsoon period	Monsoon period	During periods of closure of lake-mouth
Temperature of the atmosphere (°C)	26·0–32·3	26·2–31·1	26·6–32·0
Temperature of the substratum (°C)	27·7–32·5	26·5–30·5	27·1–30·0
Temperature of the surface water (°C)	23·0–30·5	19·5–24·5	32·5–35·4
Salinity (‰)	14·4–33·8	0·5–18·3	39·3–43·2

heavy rains during the monsoon period were not uncommon. Also, a slow but steady increase in salinity from January onwards was noticed till the end of September or even later until the onset of the next monsoon. The tidal amplitude at the mouth measured 1 m but there was a progressive decrease in the height from south to north.

3.2 *Temperature*

Temperature revealed marked variations either between that of the intertidal substratum and of the atmosphere or between monsoon and non-monsoon periods (table 1). When the mouth gets silted during south-west monsoon (June–September) due to beach drifting as happened in 1971 and 1972, both salinity and temperature shoot up in the subsequent summer post-monsoon months (January–October) (table 1) to the extent of harming much of the aquatic vegetation. If at such a time the lake is flooded with rain water, the whole of the intertidal area gets submerged below a top layer of freshwater until a new mouth is artificially cut open.

4. Vertical distribution

The intertidal substratum of the stations I–IV (figure 1) represented clean sandy to loamy nature, sometimes the loamy bottom interspersed with shell bits and also clayey to hard grassy ground composed of *Cyperus arenarius* and *C. procerus*. It was notable that the upper reaches of the eastern shore of the northern part of the lake was constituted of dry sand overlying black deoxidized layer immediately below the surface. In and around the mouth, the vertical distance between low water spring to high water spring varied from less than a metre to 2·5 metres. In contrast, in the northern region the tidal effect was so narrow and imperceptible, that the intertidal region could not be well delineated. Whereas *Ocypode cordimana* and *O. ceratophthalma* were typical terrestrial crabs, *Uca annulipes* and species of *Dotilla* and *Sesarma* were sub-terrestrial species, occurring in the intertidal region. *Matuta lunaris, Scylla tranquebarica, S. serrata* and *Portunus pelagicus* were examples of aquatic species. Station III recorded the largest number of species (21) followed by station I, harbouring 15 species. It was a regular feature to observe *O. ceratophthalma* as the only terrestrial species at a point, 23 km north of the lake-mouth. Total absence of sub-terrestrial crab species there, was conspicuous.

5. Seasonal variation

Marked variations in temperature, salinity and flooding of the intertidal region b flood water run-off between monsoon and non-monsoon periods brought i attendant changes in population and distribution of crab species. This apart occasional closure of lake-mouth during the south-west monsoon due to beac drifting adversely raised the temperature and salinity to the detriment of the faun and flora of the lake. In such a situation, the intertidal regions were fully submerge under flood water in the following flood season resulting in the upward movemen of certain species and total disappearance of others.

Six species namely *O. ceratophthalma, O. macrocera, U. annulipes, Sesarma* sp. *Metaplax distincta* and *Cardisoma carnifex* were found not affected by the change o seasons and the accompanying environmental changes. Eight species whic conspicuously dwindled in number or completely disappeared in the rainy seaso were, *M. lunaris, M. planipes, Philyra scabriuscula, Charybdis* (*Charybdis*) *orientali Thalamita crenata, O. platytarsis, Macrophthalmus* sp. and *Plaqusia depress tuberculata*. However, the rare species of *U. marionis* was observed in the monsoo and immediately after the monsoon. The rest of the 14 species did not exhibi striking fluctuation in population between the rainy and non-rainy months (tables and 3).

6. Habitats

Most of the shore crabs in Pulicat lake lived in burrows. The notable exception were species of *Matuta, P. depressa tuberculata, P. pelagicus, T. crenata* an *Macrophthalmus* sp. which resort to burrying in the soil to ward off danger. *Grapsu strigosus, Metapograpsus messor* and *Metasesarma rouxii* were often found to prefe crevices in between stones. Species of *S. serrata* and *S. tranquebarica* burrowed i the shallow intertidal area primarily for the purpose of moulting.

7. Zonation of crabs

Four zonations based on dominance of species were noticed in this lake. There wer two, *O. macrocera* zone and Scopimerinae zone in station I, only *Uca* zone i stations II and III and *O. ceratophthalma* zone in station IV. Whereas *O. macrocer* zone stretched out from high water spring to supralittoral fringe, the Scopimerina zone consisting of *D. clepsydrodactylus, Dotilla* sp. and *Scopimera pilula* extende from mean low water spring to mean high water neap. *U. annulipes* alone constitute the *Uca* zone from mean low water to supralittoral fringe and the *O. ceratophthalm* zone was observed in the supralittoral zone.

8. Penetration of crabs

All the 29 species of crabs were recorded in the vicinity of the lake-mouth a evidenced in stations I–IV and a vast majority of them started dwindling sharpl from north of station IV. Among the sub-terrestrial species, however, *U. annulipe* alone was noticed in attenuated number, a few kilometres further nort

Table 2. Density variations of the different crab species observed during 1972.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Matuta lunaris (Forskal)	1	2	2	1	0	2	1	2	2	0	0	1
Matuta planipes (Fabricius)	1	4	2	2	3	0	1	1	0	0	0	0
Philyra scabriuscula (Fabricius)	0	0	135	22	0	0	0	0	140	—	—	—
Scylla serrata (Forskal)	2	4	0	1	2	15	4	3	1	0	—	2
Scylla tranquebarica (Fabricius)	0	10	2	0	2	3	4	1	0	2	0	0
Portunus pelagicus Linnaeus	1	2	0	3	1	0	1	0	2	1	2	0
Charybdis (*Charybdis*) *orientalis* (Dana)	3	1	2	1	2	0	0	3	2	0	0	0
Thalamita crenata, Milne-Edwards	4	2	2	2	1	1	2	4	0	0	1	0
Ocypoda ceratophthalma (Pallas)	29	34	32	36	34	33	26	27	29	28	24	28
Ocypoda macrocera Milne-Edwards	24	28	32	33	31	32	34	29	29	22	21	24
Ocypoda cordimana Desmarest	13	4	4	3	5	3	3	4	2	4	1	5
Ocypoda platytarsis Milne-Edwards	1	0	1	0	0	2	0	1	0	1	0	0
Uca annulipes Latreille	33	45	50	35	69	53	54	57	63	40	37	43
Uca marionis (Desmarest)	1	0	0	0	0	0	0	0	0	1	0	0
Uca triangularis (Milne-Edwards)	12	4	0	5	4	2	0	1	3	7	8	4
Scopimera pilula Kamp	8	15	14	17	15	18	16	19	18	5	—	—
Dotilla clepsydrodactylus Alcock	—	—	15	40	38	40	60	35	41	35	—	—
Dotilla myctiroides (Milne-Edwards)	25	30	13	25	18	22	34	36	28	29	—	—
Dotilla sp.	14	13	17	21	23	20	18	17	15	26	—	—
Macrophthalmus sp.	1	3	0	0	1	2	1	—	—	1	—	—
Grapsus strigosus (Herbst)	16	8	12	6	3	4	5	8	3	5	1	2
Metapograpsus messor (Forskal)	4	2	3	5	4	1	2	4	2	2	5	0
Metasesarma rouxii (Milne-Edwards)	12	2	1	1	0	0	0	1	0	3	2	4
Sesarma (*Sesarma*) *quadratus* (Fabricius)	4	5	3	3	4	1	4	5	1	10	2	2
Sesarma (*Sesarma*) *oceanica* de Man	1	2	1	4	2	2	0	4	5	1	9	3
Sesarma sp.	5	5	10	6	13	7	10	9	4	12	12	13
Metaplax distincta (Milne-Edwards)	14	13	12	13	12	12	12	11	14	17	11	11
Plaqusia depressa tuberculata (Lamarck)	1	2	0	1	0	0	2	0	0	0	0	1
Cardisoma carnifex (Herbst)	8	7	6	9	10	11	11	11	10	12	10	8

Table 3. Population density in relation to seasonal and other effects.

X	m	$\frac{S}{X\ 100}$
1·1667	0·7993	68·51
1·1667	1·2802	109·73
24·75	50·7923	205·22
2·75	3·9396	143·26
2·00	2·7386	136·93
1·0833	0·9538	88·04
1·1667	1·1426	97·94
1·5833	1·3202	83·38
30·00	3·5590	11·86
28·1667	4·2785	15·19
4·25	2·8613	67·33
0·5	0·6455	129·09
48·1667	10·8308	22·49
0·1667	0·3727	223·61
5·0	4·3205	86·41
12·0833	6·6890	55·36
25·3333	20·2045	79·75
21·6667	11·4407	52·80
15·3333	7·7280	50·40
0·75	0·9242	123·23
6·00	4·2622	71·04
2·8333	1·5184	53·59
2·3333	3·1972	137·02
3·6667	2·3214	63·31
3·5	2·8723	82·07
8·8333	3·1842	36·05
12·6667	1·6499	13·03
0·5833	0·7592	130·15
9·4167	1·7540	18·63

Co-efficient of variation below 40 denotes distribution density is not governed by seasons; above 100 denotes distribution density governed by seasonal changes; in between 40 and 100 denotes density being governed by some other outside factors.

O. ceratophthalma was the only terrestrial species which was observed to have penetrated upto the northern most part on the eastern shore of the lake and 3 aquatic swimming crabs viz *S. serrata, S. tranquebarica* and *P. pelagicus,* enjoyed wide and uniform distribution over the entire lake.

9. Discussion

A review of the literature on the vertical distribution of brachyuran fauna from different parts of the world clearly indicates that each species prefers a specific moisture content in the substratum, on the basis of the physiological ability to withstand varying degrees of drying of the gills in the atmospheric air. In the Pulicat lake, *O. cordimana* extends to the arid, sandy regions, sometimes as far as 200 metres from the actual shore, with a water content of 8–12%. Rao (1968) reports that this species can maintain normal levels of hydration by setal uptake, since these levels are within the range of moisture content of sandy soils for which capillary forces and suction should suffice to lift interstitial moisture. *O. ceratophthalma* occupies the arid supralittoral zone and *O. macrocera*, the moist supralittoral fringe, the substrates of these regions contain about 20% of water by weight. Species of *Uca, Scopimera* and *Dotilla*, occupies varying but specific ranges in the wet intertidal belt. The portunid crabs for that matter dwell below tide level, but move back and forth with tides for feeding. This pattern of distribution undoubtedly confirms that specific moisture content of the substratum governs the vertical distribution as concluded by Dahl (1953) and Snelling (1959).

However, the adverse synergistic effect of high temperature in combination with low moisture content cannot altogether be excluded. As an illustration, the habitats of both *O. cordimana* and *O. ceratophthalma* are relatively hot and dry, compared with that of *O. macrocera*, the former species tiding over the unfavourable conditions by turning to nocturnal activity and hiding in their moist burrows during daytime, while the outside temperature is relatively higher. In contrast, the moist habitat of *O. macrocera* is not subject to serious temperature fluctuations and consequently the feeding and other activities are tuned to the rhythm of tides. Perhaps, for this reason, *O. platytarsis* which lives close to station I, prefers surf-beaten open shores to wet its gills frequently. This observation is in agreement with the statement of Dahl (1953) that the activity of the terrestrial crabs is controlled by diurnal rhythm of that of the subterrestrial species by the tidal rhythm.

Four distinct zonations were recorded in the Pulicat lake. Over and above the *Ocypoda* and *Uca* zones recorded in the Vellar estuary, Scopimerinae zone and *Ocypoda ceratophthalma* zone are observed. While *O. macrocera* and *D. clepsydrodactylus* are confined to only 1·5 km length in station I, the other two dominant species, *U. annulipes* and *O. ceratophthalma* are more widely distributed. This may be because that the former two species need clean sandy substrate with constant tidal action so as to ensure the continued existence there. This is more particularly true with *D. clepsydrodactylus* and *Dotilla* sp. since they disappear completely once the mouth of the lake gets silted resulting in the absence of tidal currents and the associated accumulation of organic substances in the once clean sand. Dahl (1953) also stresses the soil composition as a factor of great importance.

Zonation of discrete patches is conspicuous in the Pulicat lake as in the Durban Bay (Day and Morgan 1956), in the Brisbane river (Snelling 1959) and in the Vellar Estuary (Balasubrahmanyan 1966). *O. macrocera* zone and Scopimerinae zone are observed in the clean sandy region of station I and when this changed to an admixture of sand, mud and broken bits of shells as in stations II and III, the faunal components changed to *Uca* zone. Further *Ocypode* zone is observed only where there is sand. *Uca* species need clay with sand in the substratum for the construction of their non-collapsible burrows and hence they are conspicuously absent in station I, where the sandy bottom deters it (Day and Morgan 1956). The present study also confirms that patchy zonation is mainly due to the composition of the substratum.

The terrestrial species are not much affected by floods, but most of the subterrestrial species either disappear or dwindle in number during the flood season. Particularly noteworthy are *O. macrocera*, *D. clepsydrodactylus*, *S. pilula* and *Macrophthalmus* sp., and *U. annulipes* which move up to the upper reaches from their normal habitat to avoid flood water as observed by Balasubrahmanyan (1966).

The two species of *S. tranquebarica* and *S. serrata* which normally burrow in the intertidal regions for moulting were never observed there during the flood season, presumably to avoid flood water. Another factor which brings about marked physico-chemical changes is the sporadic siltation of the lake-mouth.

In the Pulicat lake only *O. ceratophthalma, P. pelagicus, S. serrata* and *S. tranquebarica* penetrate upto the northern extremities of the lake. All the subterrestrial species are confined to the southern most portion of the lake. Low salinity was reported as the limiting barrier in the penetration of intertidal crabs in the Brisbane river (Snelling 1959). In the Pulicat lake salinity and temperature are more or less uniform both at the southern and northern region at any one particular time of the year. Teal (1958) and Balasubrahmanyan (1966) are of the opinion that along with salinity, preference of habitat limit the distribution of crabs. It is pertinent to point out here that there is practically no tidal effect leading to the absence of a well defined intertidal region in the northern shores of the lake to provide the required feeding and dwelling area. Dahl (1953) and Teal (1958) concluded that food is unimportant in the distribution of those species which inhabit the sandy beaches and Georgia salt marshes respectively. However, the subterrestrial crab species depend completely on the organic matter left behind in the exposed intertidal area by the receding tide for their food supply. As such the absence of intertidal belt leads to lack of proper food supply which, in turn, perhaps leads to the absence of subterrestrial crabs in the northern portion of the lake. Over and above, the accumulation and decomposition of the drift weeds in the shores produce a large quantity of hydrogen sulphide just below surface layers. Consequently the tidal current is also important to remove the toxic substances as well as to oxygenate the area. The importance of tidal flow gains strength from the fact that in the narrow mouth region where the tidal influence is the maximum, all the 29 species of crabs are represented. Being a scavenger, *O. ceratophthalma* does not depend on the intertidal area for its food and therefore it can live wherever there is sandy supralittoral tract. The aquatic portunids, such as *S. serrata, S. tranquebarica* and *P. pelagicus* on the other hand tolerate wide fluctuations in salinity and temperature and feed on a wide variety of molluscs, crustaceans and other live or dead organisms below the tide level. Hence these may be the permitting factors for their wide and uniform distribution in the Pulicat lake.

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Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta-Heteroptera)

E T HARIDASS
Entomology Research Institute, Loyola College, Madras 600 034, India

MS received 21 December 1985

Abstract. Eggs of Triatominae and Echtrichodiinae, though identical, present distinct ultrastructural diversities in respect of collar rim, follicular pattern, position and distribution of the aero-micropylar system and the operculum, facilitating easy identification of the eggs of the species of the subfamilies. Possessing as they do, a continuous endochorionic aerostatic layer for supplying ambient oxygen to the developing embryos, they simulate other reduviid eggs. While the eggs of Triatominae are comparatively simple, being laid in exposed habitats, those of Echtrichodiinae possess additional opercular respiratory structures in view of their oviposition in specialised habitats.

Keywords. Reduviidae; Triatominae; Echtrichodiinae; eggs; chorion; operculum.

1. Introduction

Detailed studies on the egg ultrastructure have been attempted only on Harpactorinae and Piratinae (Southwood 1956; Cobben 1968; Hinton 1981; Haridass 1985b, 1986) and a wide gap still exists on this aspect in many subfamilies of Reduviidae, including Triatominae and Echtrichodiinae. Though the general egg structure of many important neotropical Triatominae, vectors of Chaga's disease are known (Usinger 1944; Abalos and Wygodzinsky 1951; Barth and Muth 1958; Ryckman 1962; Lent and Wygodzinsky 1979), very few attempts have been made to understand their ultrastructure (Beament 1946, 1947; Pick 1962; Chauvin *et al* 1973; Martin 1975). This is also true for the tropicopolitan *Triatoma rubrofasciata* (De Geer), the only species of the genus represented in India, as well as the genus and species of *Linshcosteus* represented only in India (Ghauri 1976). This, the third of the series, presents information on the ultrastructure of reduviid eggs in some species of Triatominae and Echtrichodiinae collected from southern India.

2. Materials and methods

The species of Triatominae and Echtrichodiinae studied presently are given in table 1. Methods used are similar to those described by Haridass (1985b).

3. Observation

3.1 *Oviposition*

Triatominae species deposit their eggs loosely, scattering them on the ground, under stones and boulders, or in crevices. Members of Echtrichodiinae deposit their eggs in clusters. While *Guionius nigripennis* (Fabricius) and *Ectrychotes pilicornis* (Fabricius)

Table 1. Eggs of some Triatominae and Echtrichodiinae.

	Eggs (in mm ± SD)		Number (mean ± SD)		Thickness (in μm)	
Species	Length	Width	Aeropyles	Micropyles	Chorion	Aerostatic layer
Triatominae						
T. rubrofasciata	1·71 ± 0·53	1·19 ± 0·35	126–177 155·2 ± 23·4	12–18 13·3 ± 2·94	21·43	1·74
L. costalis	1·91 ± 0·068	1·13 ± 0·037	92–119 105·57 ± 9·85	12–16 12·86 ± 1·68	12·86	1·86
L. confumus	1·82 ± 0·024	1·23 ± 0·033	98–127 110·6 ± 11·87	9–13 11·0 ± 1·58	17·14	1·78
Echtrichodiinae						
H. nigroviolaceus	3·78 ± 0·13	2·45 ± 0·95	153–168 163·6 ± 6·1	17–20 17·6 ± 1·51	22·72	2·0
G. nigripennis	2·04 ± 0·046	1·25 ± 0·046	52–68 58·8 ± 5·81	12–22 13·4 ± 7·37	15·52	1·8
E. pilicornis	1·57 ± 0·038	0·95 ± 0·036	42–45 42·0 ± 2·24	12–21 16·4 ± 3·78	9·67	1·12

glue the eggs in crevices of barks of trees, the gravid females of *Haematorrhophus nigroviolaceus* (Reuter) deposit loose egg clusters inside tunnels dug in the soil. The details of oviposition behaviour and habits of Triatominae and Echtrichodiinae have already been described (Haridass 1985a; Haridass and Ananthakrishnan 1981).

3.2 *Structure of egg*

3.2.1 *Colour, shape and size*: Eggs of Triatominae and Echtrichodiinae are creamish yellow due to the yolk within the transparent chorion. In *G. nigripennis* and *E. pilicornis*, only the operculum is distinctly whitish, while *H. nigroviolaceus* has a pale circular white band near the opercular rim. The general shape of the eggs in these subfamilies tends to be ovate with rounded base and with a simple operculum fitting into the circular neck. While the simple convex operculum in Triatominae merges imperceptibly with the general outline of the body (figures 1A,B,C; 2E), the convex operculum of Echtrichodiinae is endowed with longitudinal or oblique extremities (figures 1D,E,F; 3A,B,E).

3.2.2 *Body of the egg*: The main body of the Triatomine eggs possesses distinct hexagonal follicular markings throughout, the boundaries of these pits being more prominent in *Linshcosteus* species than in *Triatoma rubrofasciata* (De Geer) (figures 2A,D,G,H). In Echtrichodiinae the follicular sculptures are prominent only near the neck, becoming feeble and insignificant towards the base of the eggs. Unlike those of *H. nigroviolaceus*, the eggs of *G. nigripennis* and *E. pilicornis* are covered with female accessory secretions during oviposition and the secretions are deposited as 5–7 long ribs extending from opercular tip to the base (figure 1E). They considerably obscure the surface and serve to glue the eggs to the crevices of tree trunks.

Only the exo- and endochorionic layers could be observed in the body of the eggs of both subfamilies. The chorion in all the eggs, is of uniform thickness throughout, except at the neck where it is thicker. The relative thickness of the egg shell in different species is tabulated (table 1). The innermost part of the endochorion has an aerostatic layer, visible only at high magnification, varying between 1·12–2·2 μm thick in different species. It is distributed uniformly throughout the inner lining of the eggs and commences immediately from below the lower limits of the sealing bar, at which point it is wider than the rest of the chorion (figures 1 A1,D1; 3F). The aerostatic layer is separated from the endochorion by small piller-like struts, enclosing a network of continuous air-spaces between them, thereby establishing a connection between the ambient air and the interior of the egg through the aeropylar system.

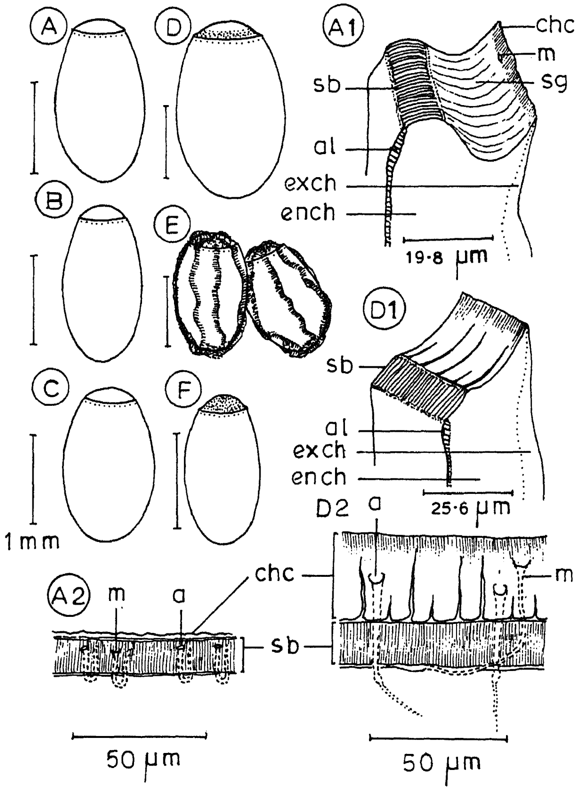

Figure 1. Eggs of Triatominae and Echtrichodiinae. **A, A1 and A2.** *T. rubrofasciata.* **A.** Entire egg with operculum. **A1.** LS of collar region showing collar rim, sealing bar, and spermatic groove with micropyle opening. **A2.** Collar region showing sealing bar, aeropyles, and micropyles. **B.** *L. costalis.* Entire egg with operculum. **C.** *L. confumus.* Entire egg with operculum. **D, D1 and D2.** *H. nigroviolaceus.* **D.** Entire egg with operculum. **D1.** LS of collar region showing collar rim, with supporting ribs and sealing bar. **D2.** Collar region showing sealing bar, aeropyles and micropyles. **E.** *G. nigripennis.* Entire eggs covered with female accessory secretions. **F.** *E. pilicornis.* Entire egg with operculum. (a, Aeropyles; al, aerostatic layer; ch c, chorionic collar rim; ex ch, exochorion; en ch, endochorion; m, micropyle; sb, sealing bar; sg, spermatic groove).

3.2.3 *Chorionic collar*: Anteriorly the reduviid egg is marked by a neck or collar which appears slightly constricted than the rest of the body. There is marked variation in the structure of the collar region in the two subfamilies. In the Triatominae the neck has a circular band-like short collar rim composed of exochorion (figures 1 A1; 2B) and an inner endochorionic, convex or slanting, ridged and grooved ring of sealing bar facing the lumen of the eggs (figures 1 A1; 2B,C,F). The upper margin of the collar rim and the extremities of the operculum enclose between them a spermatic groove (figures 2 A,B,C,E,F). The collar rim and the spermatic groove of *Linshcosteus costalis* (Ghauri) and *L. confumus* (Ghauri) are reduced and not as prominent as in *T. rubrofasciata*. But in other aspects eggs of these species present a uniform picture. The chorionic sealing bar receives the concave or slanting ring of ridges and grooves around the inner periphery of the operculum enabling the latter to fit perfectly into the circular opening of the neck (figure 2E).

The collar region of Echtrichodiinae is distinguished by an outer exochorionic collar rim, extending as short vertical projections around the neck which becomes clearly visible after eclosion of eggs. The collar rim measures an average of 38·57 μm in *H. nigroviolaceus*, 37·14 μm in *G. nigripennis* and 18·57 μm in *E. pilicornis*. In all the species this rim is supported by numerous, vertical, short or long inner tapering ribs (figures 1 D1; 3C). The endochorionic sealing bar, oriented either horizontally or obliquely to the long axis of the egg, has its surface marked by alternating grooves and ridges (figures 1 D1; 3C). Similar grooves and ridges of the opercular rim enable the latter to fit snugly into the chorionic collar. The width of the sealing bar is narrow in *E. pilicornis* (12·86 μm), moderate in *G. nigripennis* (17·14 μm), and wide in *H. nigroviolaceus* (18·57 μm). The upper limits of the collar rim of these eggs are flushed with the extremes of the operculum and as such a spermatic groove is totally wanting. During eclosion of the Triatominae and Echtrichodiinae eggs, the simple contacts between the collar rim and the operculum are removed all around by the pressure exerted by the escaping embryo, enabling the latter to be pushed aside like a lid of a jar.

3.2.4 *Aeropyles and micropyles*: Like the collar, the structure and the position of the aeromicropylar system of Triatominae and Echtrichodiinae also differ, though a uniform pattern is found in the representative species of each subfamily. In Triatominae the aeropylar number varies from 92–177 (table 1). The external aeropylar openings situated at the middle level or at the extremes of the collar rim band all around (figures 1 A2; 2A,C,H), are easily distinguished by their wide canals (2·9 μm in *T. rubrofasciata*; 3·2 μm in *L. costalis*; 1·8 μm in *L. confumus*). Also, these openings are located on a higher level (6·47–8·57 μm) than the external micropylar openings (figure 1 A1). The aeropyles take a vertical course through the chorion, but make a 'U' turn in the endochorion and terminate as internal openings below the sealing bar into the aerostatic layer (figure 1 A2). The micropyles in all these species start from near the chorionic rim, but their funnel-shaped openings face the lumen of the spermatic groove (figures 1 A1,A2; 2B) and hence remain invisible during observation of the collar surface. Like the aeropyles, the micropyles also take a vertical course and make a 'U' turn in the endochorion, but taper down as narrow canals to open ultimately into the lumen of the egg through small openings (1·4–2·14 μm), slightly above the level of the inner aeropylar openings (figures 1 A2; 2B).

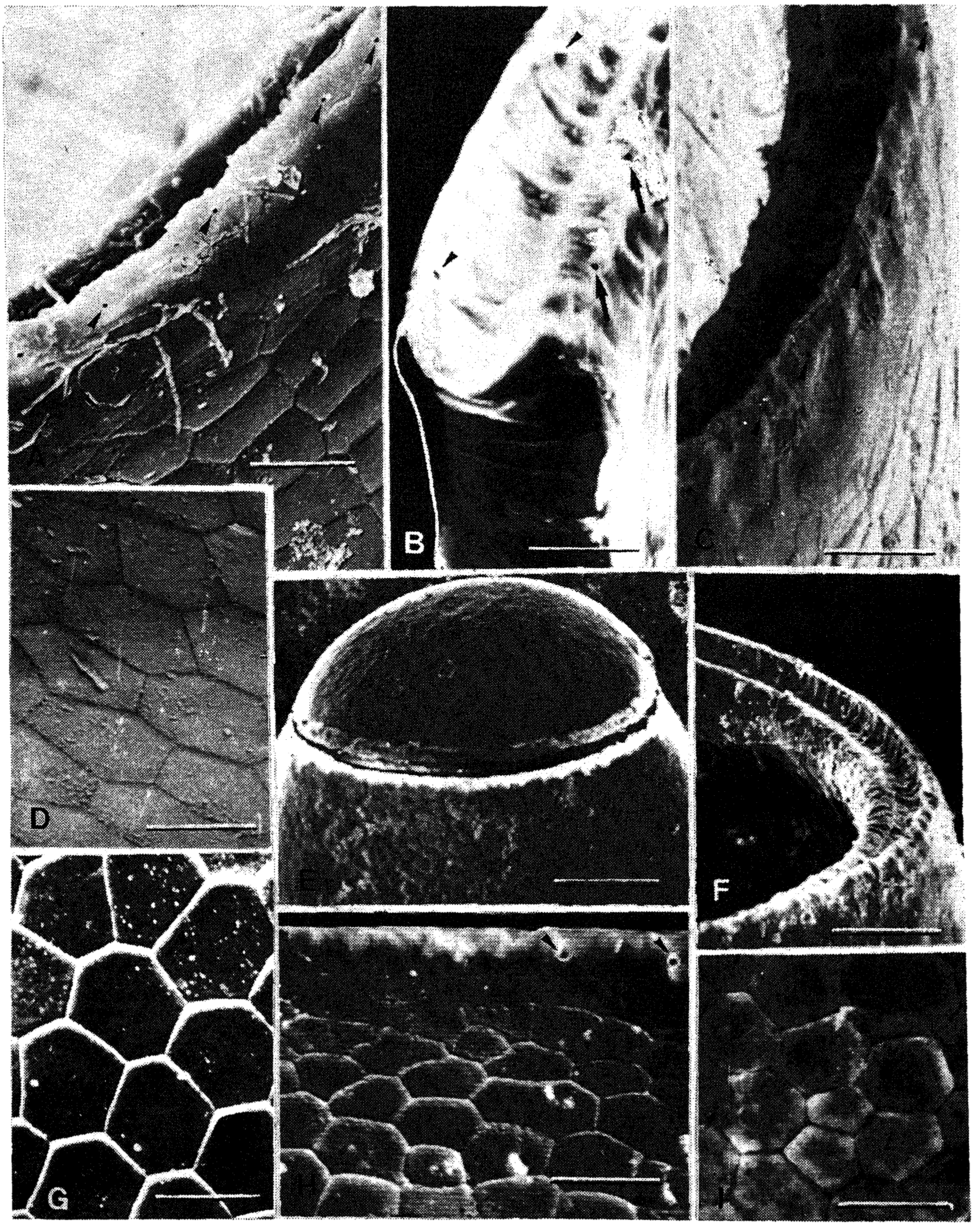

Figure 2. Scanning electron micrographs of Triatomine eggs. **A–D.** *T. rubrofasciata*. **A.** Collar region showing spermatic groove and aeropyles (arrows) (× 22·2 μm). **B.** Surface view of the spermatic groove showing outer micropylar openings (small arrows), sealing bar and the inner micropylar openings (long arrows) (× 15·8 μm). **C.** Collar region showing the sealing bar, outer aeropylar openings (arrows), and the spermatic groove (× 20·3 μm). **D.** Chorionic follicular markings (× 18·2 μm). **E–G.** *L. costalis*. **E.** Anterior part of the egg with operculum (× 153·9 μm). **F.** Collar region showing the sealing bar and the spermatic groove (× 51·3 μm). **G.** Chorionic follicular markings (× 18·9 μm). **H and I.** *L. confumus*. **H.** Collar region showing the aeropyles (arrows) and the follicular markings (× 20·25 μm). **I.** Follicular markings on operculum (× 15·39 μm).

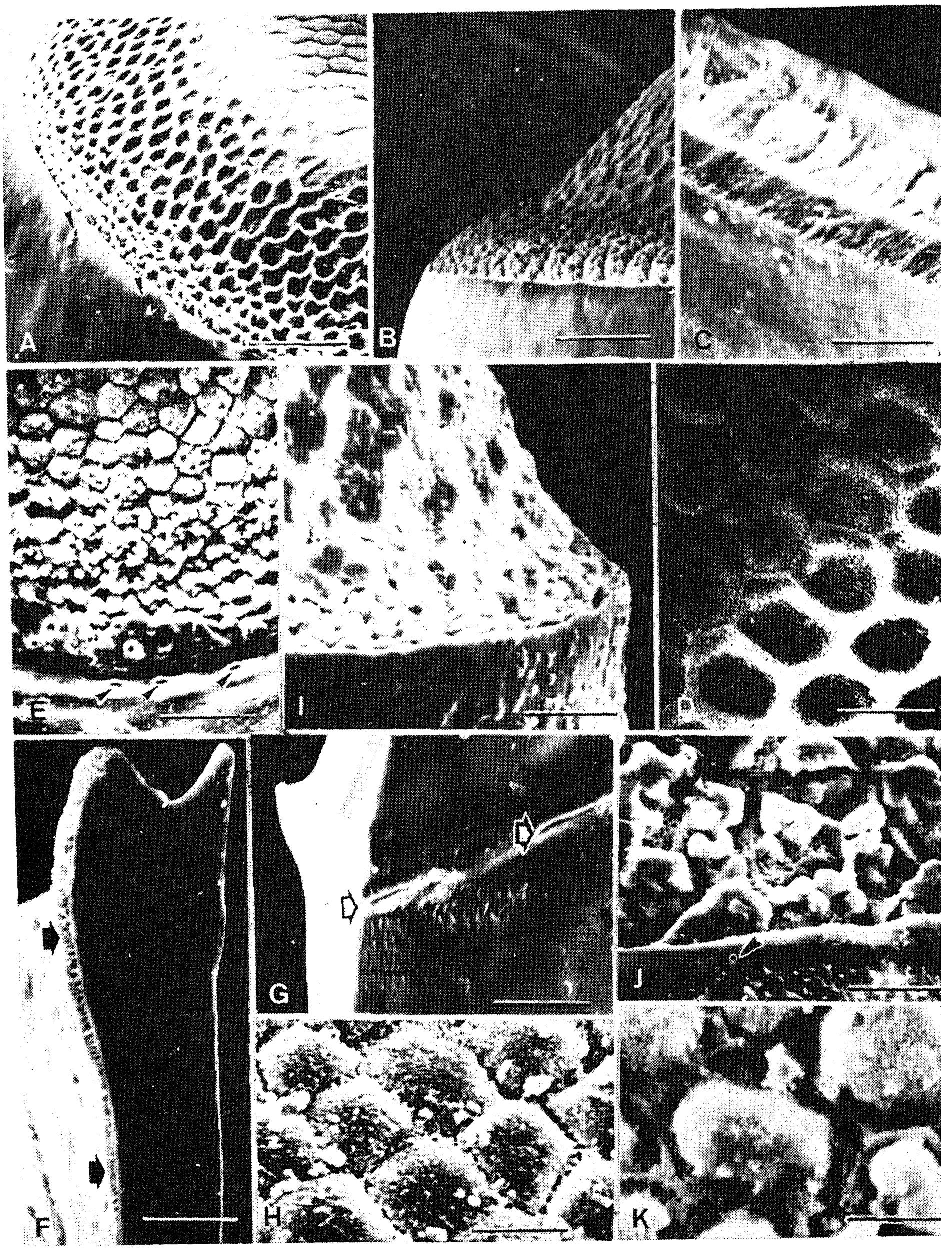

Figure 3. Scanning electron micrographs of echtrichodine eggs. **A–D.** *H. nigroviolace* **A.** Collar region showing the aeropyles (arrows) and the operculum (× 60 µm). **B.** Anter part of the egg with the operculum (× 61·5 µm). **C.** Inner view of the collar region showi the collar rim and the sealing bar (× 20·5 µm). **D.** Follicular markings on the operculu (× 20·5 µm). **E–H.** *G. nigripennis.* **E.** Collar region showing the collar rim with the aeropy (arrows) and the operculum (× 40·5 µm). **F.** LS of collar region showing endochorio aerostatic layer (× 15 µm). **G.** Inner view of the collar region showing the inner micropy openings (arrows) (× 19·5 µm). **H.** Follicular markings on the operculum (× 15 µm). **I-** *E. pilicornis.* **I.** Anterior region of the egg with operculum (× 30·4 µm). **J.** Collar region w the aeropyles (arrows) (× 14·3 µm). **K.** Follicular markings on the operculum (× 9·5 µm)

In Echtrichodiinae, the external openings of both aeropyles and micropyles are located on the chorionic collar (figures 3A,E,J), the circular wide openings of the former being located almost at the same or slightly at a higher level than the latter. The aeropylar canals make a vertical course through the chorion and open into the aerostatic layer through wide inner openings below the sealing bar. Small tapering canals also extend from these openings into the aerostatic layer (figure 1 D2). The width of the aeropylar canals measures an average of 3·4, 4·3 and 1·14 μm in *H. nigroviolaceus*, *G. nigripennis* and *E. pilicornis*, respectively. The micropyles of Echtrichodiinae are well defined and easily recognised by their funnel-shaped openings and at the level of the inner aeropylar openings they bend at right angles and run a distance parallel to the sealing bar and finally terminate as shallow grooves (figures 1 D2; 3G). When viewed from the anterior end, the micropyles exhibit a clockwise arrangement. The number of aeropyles and micropyles of Triatominae and Echtrichodiinae are given in table 1.

3.2.5 *Operculum*: The simple operculum of Triatominae and Echtrichodiinae presents sufficient structural variations to distinguish between the eggs of the two subfamilies. In the former the operculum is a simple convex disc, whose outline merges imperceptibly with the general shape of the egg. The opercular surface exhibits simple hexagonal follicular pits as seen over the egg surface without tubercles, projections, etc., but having numerous minute air-pores in the inter-follicular boundaries (figure 2I). The operculum of Echtrichodiinae is hat-like with a central convex disc possessing horizontal or oblique extremities (figures 1D,E,F; 3A,B,I) that continue with the general outline of the egg. The opercular surface-ultrastructure shows considerable variation in the 3 species. In *H. nigroviolaceus* the apical 3rd of the operculum has smooth hexagonal follicular markings and centrifugally the lower boundaries of the pits become greatly thickened giving the appearance of overlapping tiles. On the horizontal part, these markings are transformed into hexagonally arranged, vertical knob-like projections with blunt tips, enclosing a small space between them. The projections as well as the surface and the interfollicular boundaries are highly porous giving a spongy appearance (figures 3A,B,D). In *G. nigripennis* the entire opercular surface is marked by numerous polygonal follicular markings, the central part of each being raised into a convex spongy projection. Such projections become more pronounced towards the opercular rim (figures 3E,H). The operculum of *E. pilicornis* also shows similar structures, but the spongy projections are restricted to the central part of each follicular area, so that the inter-follicular boundaries are wider and more prominent with numerous air-pores (figures 3I,J,K). In all the 3 species minute air canals also traverse the opercular wall, connecting the spongy outer surface with the lumen of the egg. The exo- and endo-chorionic differentiations as well as the inner aerostatic layer seen in the body, are absent in the operculum of both these subfamilies.

4. Discussion

The eggs of Reduviidae are unique in the structure of their operculum, chorionic collar, and the aeromicropylar systems. Analysis of the eggs of different subfamilies, particularly Piratinae (Haridass 1985b) and Harpactorinae (Southwood 1956;

Cobben 1968; Salkeld 1972; Hinton 1981; Haridass 1986) have shown that the egg structures are useful in assigning characters atleast at the subfamily level. The apparent simplicity of the Triatominae and Echtrichodiinae eggs has prompted Southwood (1956) to conclude that the eggs of these are similar. But the present study of the egg characteristics has shown clearly the differences between them, proving once again the usefulness of the egg ultrastructure in the recognition of various subfamilies.

Generally the eggs of Triatominae, like those presently examined, are ovate having almost an imperceptible neck with the convex operculum fitting into it (Usinger 1944; Abalos and Wygodzinsky 1951; Ryckman and Ryckman 1967; Lent and Wygodzinsky 1979). A distinctly constricted neck is seen only in *Rhodnius prolixus* Stal, *Psammolestes arthuri* (Pinto) and *Cavernicola pilosa* Barber among Triatominae (Foratini and Barata 1947; Lent and Wygodzinsky 1979). The eggs of Triatominae are also characterized by a wide or narrow spermatic groove and a band-like collar rim with aeropylar openings on the surface or at the extremities, the micropylar openings being situated inside the spermatic groove. In addition the external openings of the aeropyles and micropyles are located at different levels at the collar rim; both the aeropyles and micropyles make 'U' bend in the chorion. The hexagonal follicular markings on the operculum are without surface projections and granulations. But there are reports in other Triatominae of the occurrence of small pits within follicular hexagons as in *Triatoma infestans* Klug and in *T. maculata* (Erichson) or on the boundaries of the hexagons as in *T. proteinus*, *Panstrongylus megistus* (Burmeister) and *Eratyrus mucronatus* Stal, or deep single pit within the hexagons as in *Rhodnius prolixus* Stal and *Psammolestes arthuri* (Pinto) (Chauvin *et al* 1973; Lent and Wygodzinsky 1979; Hinton 1981). The follicular markings of the chorion and the operculum of Triatominae have been shown to be species specific (Ryckman 1962; Lent and Wygodzinsky 1979) and Ryckman (1962) has shown the instances of deposition of smooth and rough surfaced eggs by two geographically separated populations of *Triatoma protracta* complex. No species specific characters appear to be evident in *Linshcosteus*, but their regular hexagonal follicular sculpture and deep follicular boundaries can be easily identified from the trapezoid follicular markings and shallow boundaries of *T. rubrofasciata*.

The eggs of Echtrichodiinae are characterised by short, vertical collar rim without spermatic groove. Both the aeropyles and micropyles have their external openings located on the outer surface of collar rim, the oval or circular openings of the former always being located on the edges of the collar rim, only slightly above or below the funnel shaped openings of the latter. While the aeropyles take a verticle inward course into the chorion, the micropyles are 'L' shaped and arranged clockwise around the collar. A similar arrangement has been reported for *Echtrichodia antennalis* Stal (Cobben 1968). Moreover, these eggs have distinct follicular markings restricted to the narrow region below the neck, the markings becoming less prominent towards the basal parts. The opercular surface of these eggs vary considerably, the sculptures are characterised by highly porous and spongy areas and boundaries, with minute pore-canals leading to the interior of the egg. This kind of additional respiratory structure for these eggs is only natural, since in Echtrichodiinae the oviposition of loose egg clusters is within tunnels dug into the soil as in *H. nigroviolaceus*, or gluing egg masses inside crevices of tree trunks as in *G. nigripennis* and in *E. pilicornis* (Haridass 1985a). The common method of oviposition in Triatominae is simple and eggs are deposited loosely in dry exposed areas under rocks and boulders, in crevices or in bird coops etc (Ryckman 1962; Lent and Wygodzinsky 1979; Haridass and Ananthakrishnan 1981; Haridass 1985a) and only *P. arthuri* among Triatominae, is known to frequently lay masses of subcylindrical eggs glued together (Lent and Wygodzinsky 1979).

The aerostatic layer of reduviid eggs, serving to distribute the ambient oxygen to the developing embryos, was believed to be restricted only to the neck region, in both Triatominae and Echtrichodiinae (Southwood 1956). But the present study has shown that this is distributed throughout the inner surface of these eggs. Similar distribution of the aerostatic layer is also seen in Piratinae and Harpactorinae (Haridass 1985b, 1986). The presence of a large continuous canal in the chorion running parallel to the aerostatic layer and receiving the aeropyles from the follicular surfaces, as reported in *T. infestans* (Chauvin *et al* 1973), is not observed in any egg. The distribution of the aerostatic layer as a uniform layer throughout the inner surface of egg appears to be a common feature for all reduviids.

There is wide variation in the number of aeropyles, not only in different species, but also among different eggs of the same species (e.g. *T. rubrofasciata*—126–177). Variation in the number of micropyles is also common. As pointed out by Beament (1947), such variations are due to the reduction of these structures in the eggs that are laid at the end of the oviposition cycles. The number of micropyles has been reported to be not more than 15 in Reduviidae (Cobben 1968), but this is not in agreement with the present study where the number was as high as 22 in Echtrichodiinae and 17 in Triatominae (table 1). Similar higher number was also reported for the eggs of Piratinae (Haridass 1985b).

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Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets

B VENKATESH*, A P MUKHERJI, P K MUKHOPADHYAY and P V DEHADRAI**

Central Inland Fisheries Research Institute, Barrackpore 743 101, India
*Present address: National Bank for Agriculture and Rural Development, Bangalore 560 001, India
**Present address: Fisheries Development Commissioner, Ministry of Agriculture and Cooperation, New Delhi 110 001, India

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Abstract. Specific growth rate and protein efficiency ratio were highest in *Clarias batrachus* (Linn.) fed with Halver's diet followed by fish fed with fishmeal, dried silkworm pupae, meatmeal and groundnut oilcake in decreasing order. Protein synthesis in liver was maximum in fish fed with standard test diet followed by those fed with silkworm pupae, fishmeal, meatmeal and oilcake fed fish. Intestinal protease activity was unaltered in all fishes except those on oilcake diet. Level of total serum protein of different groups of test fishes were comparable. Growth of fish and serum Ca : P ratio were positively correlated ($P < 0.05$). The results suggest that animal protein component is a better source of protein in the diet of *Clarias batrachus* for satisfactory growth and that fishmeal and silkworm pupae can be incorporated in the operational diet with advantage.

Keywords. Nutrition; *Clarias batrachus.*

1. Introduction

Utilisation of protein by fishes is influenced by protein quality and gross protein level of the diet (Steffens 1981). In this paper, we report the influence of diets with different protein sources on the growth and metabolism of the tropical catfish *Clarias batrachus* (Linn.).

2. Materials and methods

Healthy specimens of *C. batrachus* (weight ~20·5 g, stocking density 15 per cistern) were fed with 5 different feeds (fishmeal diet, meatmeal diet, silkworm pupae diet, groundnut oilcake diet and Halver's standard diet, see table 1 for composition) at 8% of body weight per day for 8 weeks. The composition of the feed was adjusted to 25% protein content.

Individual fish was weighed every week and percentage gain over initial weight, conversion ratio, protein efficiency ratio and specific growth rate were calculated. At the end of the study period, physiological parameters (see below) were measured in the experimental fishes. The water temperature during the study ranged from 24·0–28·0°C.

For measuring the intestinal protease activity, the scrappings from the intestine were processed as per the method described by Mukhopadhyay *et al* (1978) and the proteolytic hydrolysis compounds were assayed following the method of Moore and

Table 1. Composition (in %) of experimental diets[a]

	Fishmeal diet (a)	Meatmeal diet (b)	Silkworm pupae diet (c)	Oilcake diet (d)	Halver's standard diet (e)
Bulk a,b,c,d or e	60·23	28·94	58·38	53·90	100·00
Rice bran	9·77	41·06	11·62	16·09	—
Wheat flour	29·00	29·00	29·00	29·00	—
Yeast	1·00	1·00	1·00	1·00	—
Moisture	15·64	15·58	16·62	18·16	50·00
Crude protein	24·73	25·48	24·73	24·30	25·00
Ash	19·75	11·83	4·68	6·75	2·00
Crude fat	6·50	4·97	10·60	7·54	4·50
Crude fibre	3·46	8·62	3·64	7·52	4·00
Nitrogen Free Extract (NFE)	29·92	33·88	39·73	35·72	14·50

[a]Proximate composition of all test diets except Halver's was estimated analytically.

Stein (1948). The protease activity was expressed as Δ absorbance at 570 nm/mg protein/hr.

Protein synthesis in liver was measured as incorporation of L-lysine-U^{14}C (specific activity 120 mCi/m mol; obtained from Bhabha Atomic Research Centre, Trombay, Bombay). The fish was injected with the isotope at the rate of 10 μCi/100 g body weight, sacrificed after 2 hr and liver separated. Protein was precipitated at a final tricarboxylic acid concentration of 5%. The precipitate was processed and the radioactivity was counted in an automatic liquid scintillation counter and expressed as cpm/10 mg liver tissue.

Serum protein was estimated by the method of Lowry *et al* (1951).

Blood serum calcium and phosphorus were estimated by Clark-Collip modification of the Kramer-Tisdall method and Fiske and Subba Row method, respectively as described by Oser (1960).

3. Results

Fish fed on Halver's standard diet for 56 days showed 179·1% increase over initial weight while those fed on fishmeal diet, silkworm pupae diet, meatmeal diet and groundnut oilcake diet recorded 67, 65·9, 36 and 6·2% increase respectively (figure 1). The best conversion ratio was obtained with Halver's standard diet followed by fishmeal diet, silkworm pupae diet, meatmeal diet and groundnut oilcake diet. Protein efficiency ratio and specific growth rate of fish fed on different diets also followed similar trends (table 2).

Table 3 presents details of physiological parameters recorded from fishes fed with experimental diets. Fish fed with oilcake and meatmeal diets showed significantly lower levels of intestinal protease than those fed with other diets ($P < 0{\cdot}1$). The rate of protein synthesis varied with diets and was highest in fish fed with standard diet and lowest in fish fed with oilcake diet. Protein synthesis rates in fish fed with silkworm pupae and fishmeal diet were significantly higher than that in fish fed with meatmeal diet.

Between the different groups of fishes the serum Ca : P ratios were significantly

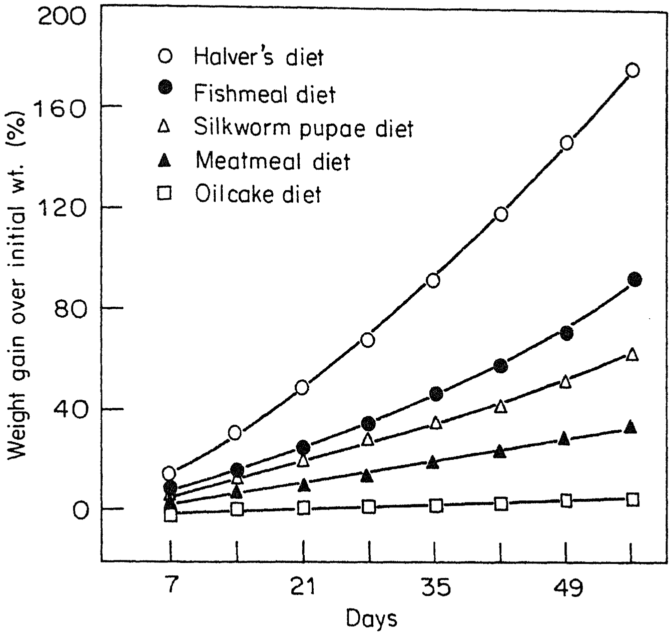

Figure 1. Weight gain of *C. batrachus* fed with different diets.

Table 2. Effect of different diets on growth and conversion efficiency of *C. batrachus*.

	Halver's standard diet	Fishmeal diet	Meatmeal diet	Silkworm pupae diet	Oilcake diet
Conversion ratio	3·36	5·19	10·98	5·31	39·67
Protein efficiency ratio	0·82	0·61	0·38	0·59	0·17
Specific Growth Rate[a]	1·7	1·2	0·6	1·0	0·1

[a] Expressed as growth per day as percentage of body weight (α)

$$W_t = W_o\left(1+\frac{\alpha}{100}\right)^t$$

different and related to the growth responses of fish. Analysis of the blood revealed no significant variation in the levels of serum protein in fish fed with different diets.

4. Discussion

The 5 feeds used in the present study were isonitrogenous (25% crude protein) and as such the differences in the responses between the various groups of fish may be related mainly to the differences in protein quality. The lower than the normal growth rate (recorded under earthern pond conditions) may be related to the lower water temperatures (24–28°C). Halver's standard diet (protein source casein and gelatin) gave the highest growth and conversion and protein efficiency ratio. Apparently, the proteins in fishmeal and silkworm pupae diets were utilised equally by the fish while the meatmeal diet was poorly utilised. The plant protein based

Table 3. Effect of different diets on certain physiological parameters of *C. batrachus*.

	Halver's diet	Fishmeal diet	Meatmeal diet	Silkworm pupae diet	Oilcake diet
Intestinal protease activity (ΔOD 570/mg protein/h)	12·0 ± 0·03	12·52 ± 0·30	10·14 ± 0·61	12·89 ± 0·60	8·02 ± 0·15^{a}
Aminoacid (l-lysine-U^{14}C) incorporation (cpm/10 mg liver tissue)	375·53 ± 2·79	146·30 ± 2·58	86·30 ± 1·86^{b}	166·24 ± 3·83	13·78 ± 1·36^{b}
Serum protein (g/100 ml)	4·01 ± 0·004	4·04 ± 0·0011	4·12 ± 0·0374	3·80 ± 0·2160	4·01 ± 0·0838
Serum Ca: *P* ratio	0·079 ± 0·0008	0·075 ± 0·0041	0·058 ± 0·0015	0·071 ± 0·0009	0·053 ± 0·0015^{c}

Values are expressed as Mean ± S.E. of 5 observations. Levels of significance are indicated for values on the same row.
$^{a}P < 0{\cdot}1$; $^{b}P < 0{\cdot}01$; $^{c}P < 0{\cdot}05$.

oilcake diet resulted in very low growth rate and poor conversion and protein efficiency ratios. A loss in weight of fish fed with this diet was recorded during the first week. Protein from groundnut oilcake appears to be nutritionally unsuitable for growth of *C. batrachus.*

High protease activity has been reported in the intestine of *C. batrachus* which exhibit predilection for carnivorous diet (Mukhopadhyay *et al* 1978). The present study shows that the level of protease activity in fish fed with predominantly animal protein diet was higher than that in fish fed with a total plant protein diet. While the influence of dietary protein level on the protease activity in fishes is well documented (Kawai and Ikeda 1973; Mukhopadhyay *et al* 1978; Steffens 1981), there is no conclusive evidence to illustrate the relationship between the source of dietary protein and the level of protease activity. Such variations in enzyme activity may be related to the structure of protein and the duration of retention of feed in the intestine which in turn depends on the fibre content and physical consistency of the diet.

Protein synthesis rates in fishes fed with different diets followed the same trend exhibited by the growth rate of the fishes. This indicates the probable role of the composition of diets on protein synthesis and also the nutritive value of their ingredients.

The serum Ca-*P* ratios of fish fed with different diets are correlated with growth and conversion efficiency of fish. This indicates some limited application of the serum Ca-*P* ratio for evaluating the nutritional background/history of fishes under experimental conditions.

Steady levels of serum protein in fish with varied rates of protein synthesis indicate the inherent ability to maintain constant serum protein levels even under varied nutritional conditions. This is similar to the ability of the toad fish, *Opsanus tau* to maintain a certain level of circulating plasma protein under conditions of increased protein synthesis in the liver (Hashemeyer 1973).

Superiority of fishmeal over other ingredients in the practical diets of various fishes has been reported (Andrew and Page 1974; Viola 1975; Fowler and Banks 1976; Davis and Stanley 1978). In the present experiment, comparison of the growth rate and other physiological parameters of fish fed with fishmeal diet and silkworm pupae diet indicates that the nutritional value of silkworm pupae compares well with that of fishmeal but the better growth response may be related to higher fat and carbohydrate contents and protein quality. In *Catla catla,* silkworm pupae diet was found to result in a better growth rate than that by fishmeal diet (Jayaram and Shetty 1980). Thus, silkworm pupae, a waste product in silk industry, can replace fishmeal in formulated fish diets.

5. Conclusion

Fishmeal and silkworm pupae are better sources of protein in the diet of *C. batrachus* than meatmeal and groundnut oilcake. The conventional diet used in carp culture, a mixture of oilcake, rice-bran and wheat flour, was a nutritionally poor diet for *C. batrachus.*

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Trypanosoma evansi infection and the influence of some hormones on haematological indices in albino rats

M LEMALATHA, L NARASIMHA RAO and M KAMESWARI
Department of Zoology, Kakatiya University, Warangal 506 009, India

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Abstract. Changes in the haematological indices like haemoglobin, mean corpuscular haemoglobin, colour index, iron, total red blood corpuscles, white blood corpuscles and blood glucose in albino rats during *Trypanosoma evansi* infection and the influence of some hormones like hydrocortisone, insulin and thyroxine on these changes have been investigated.

Keywords. Haematological indices; *Trypanosoma evansi*; hormone changes.

1. Introduction

Haemoflagellates cause several alterations in the haematological indices. The degree of changes in these is of significance in understanding the degree of pathogenecity. Therefore, from veterinary and medical point of view, they are of much importance and have received much importance in recent years. Haematological changes in trypanosomiases were reported by a number of workers (Yorke 1910; Nausa and Yorke 1911; Fenyvessy 1926; Kligler *et al* 1929; Andrews *et al* 1930; Muniz and Freitas 1945; Moulder 1948; Von Brand *et al* 1955; Edwards *et al* 1958; Sen *et al* 1959; Richardson and Kendall 1963; Srivastava 1965; Naylor 1971; Jatkar *et al* 1973; Mackenzie and Cruikshank 1973; Woodruff *et al* 1973; Jatkar and Mohan Singh 1974; Jennings *et al* 1974; Mamo and Holmes 1975; Woo and Kobayashi 1975; Kobayashi *et al* 1976; Rickman and Cox 1979; Damayanthi 1982).

Effect of hormones on the haematological changes during trypanosome infection have also been studied (Knowles and Dasgupta 1927; Sherman and Ruble 1967; Seed *et al* 1972).

2. Material and methods

Albino rats of Wistar strain were procured from the National Institute of Nutrition, Hyderabad and maintained in the laboratory under uniform environmental conditions and diet for about a week. *Trypanosoma evansi* were collected from the naturally infected cattle and maintained in the laboratory by syringe passage on every 3rd day into albino rats. A constant dosage of parasites (25×10^6 trypanosomes per ml of blood) was inoculated intraperitoneally into experimental animals. Experimental studies were carried out when the parasitemia was at peak.

The hormone dosages used in all the studies were 0·33 mg of hydrocortisone (obtained from Russel Pharmaceuticals of India, Bombay), 1·33 units of insulin (obtained from Boots Company, Bombay) and 0·08 mg of thyroxine (obtained from Cadilla chemicals, Ahmedabad) per 100 g of body weight. Each dose was administered starting from two days prior to infection and throughout the period of experiment everyday. From different hosts, blood samples were obtained by cutting the tip of the tail and various parameters were assayed by the following procedures.

Haemoglobin content was determined colorimetrically by acid haematin method of Cohen and Smith (1919). The haemoglobin (Hb) percentage was calculated by taking 15 g of haemoglobin per 100 ml blood as 100%.

The mean corpuscular haemoglobin (MCH) was determined by the equation

$$\mathrm{MCH} = \frac{\text{Hb in g/100 ml} \times 10}{\text{Red cells in million/cubic mm}}$$

Colour index (CI) which indicates the proportion of Hb present in each red blood cells (RBCs) was calculated by the formula,

$$\mathrm{CI} = \frac{\text{Percentage of Hb}}{\text{Percentage of RBCs}}$$

Total iron content was estimated by the method of Wong San Yin (1923). Total number of red blood cells and white blood cells (WBCs) were counted separately by a haemocytometer with double neubauer ruling. The percentage of red cell count of the sample was determined by taking a count of 5 million red cells per cubic mm as 100%.

Blood glucose was determined following the method of Folin and Wu (1929).

3. Results

3.1 *Hb*

The Hb content of the control rat is 96%. It is reduced by about 30% in infected rats. Mere treatment with hydrocortisone and insulin decreased the Hb level by about 7 and 2%, respectively, whereas thyroxine increased it by 4%. In hydrocortisone, insulin and thyroxine treated and infected albino rats the Hb content decreased significantly by about 40, 29 and 23%, respectively.

3.2 *MCH*

The MCH of control rats is 6·47 ± 0·59 μg/100 ml of blood and it increased by 165% during *T. evansi* infection. MCH values decreased by about 6% in hydrocortisone treatment but increased in insulin and thyroxine treated rats by about 7 and 15%, respectively. The MCH level significantly increased by about 225% and 31% in hydrocortisone and thyroxine treated and infected groups respectively; but decreased by about 5% in insulin treated and infected group.

3.3 *CI*

The CI of control animals is 0·21 ± 0·02. It raised by 171·42% upon *T. evansi* infection. Mere treatment by hydrocortisone reduced it by 5% but insulin and thyroxine insignificantly increased by about 10 and 19%, respectively. But CI in the hydrocortisone treated and infected group has risen significantly to 233·33%. This increase is insignificant in insulin and thyroxine treated and infected groups.

3.4 *Iron*

Iron content of control rats is 47·03 ± 2·71 mg/100 ml of blood and it decreased by about 30% in *T. evansi* infected rats. Hormone treatment insignificantly lowered the iron content but trypanosome infection of treated rats significantly decreased it.

3.5 *RBCs*

RBC count of normal rats is 22·8 ± 2·58 millions per cubic mm of blood and the count decreased by about 70% upon *T. evansi* infection. In hormone treated groups the count decreased by about 4% but the infection of treated rats lowered it significantly by about 77, 38 and 34% in the case of hydrocortisone, insulin and thyroxine, respectively.

3.6 *WBCs*

Leucocyte count of control albino rats was 2218 per cubic mm of blood and decreased by 194% upon *T. evansi* infection. In the albino rats, treated with hydrocortisone, insulin and thyroxine, WBC count was lowered by 3, 16 and 7%, respectively. But the infection of treated rats resulted in their significant rise by about 303, 179 and 171% when treated by hydrocortisone, insulin and thyroxine, respectively.

3.7 *Blood glucose*

The blood glucose content of the control albino rats is 145 mg/100 ml of blood. It decreased by about 58% upon *T. evansi* infection. In all hormone treated rats, the blood glucose content decreased and showed a further decrease upon trypanosome infection.

4. Discussion

The decrease in Hb, iron, RBCs and blood glucose and increase in leucocytes, MCH and CI in infected albino rats suggests the drastic pathogenic effect of the presence of *T. evansi* infection.

Anaemia is common feature in mammalian trypanosomiasis, especially in the *Brucei evansi* group. It may be due to the erythrocyte destruction (Mamo and Holmes 1975; Naylor 1971) or unproportional erythropoietic compensation (inefficient Hb synthesis) resulting in the lowered Hb content in infected rats. The haemoglobin content decreased in hydrocortisone and insulin treated rats whereas thyroxine treatment resulted in slight increase. Generally hormone treatment reduces the resistance and this effect is more in hydrocortisone treated and infected group, but less in thyroxine treated and infected groups. Insulin has less effect upon haemoglobin change.

The rise in MCH values upon *T. evansi* infection is possible when the number of RBCs is drastically decreased, but the Hb values showed lesser decrease. Consequently, the ratio of haemoglobin to RBCs is more and each red cell contains more amount of Hb. The number of calls may be less, but each one lodges relatively higher proportion of Hb. Presumably, the globin part from the haemoglobin of destroyed RBCs is reutilized in the synthesis of new RBCs.

The fall in MCH values of hydrocortisone treated rats indicates that relatively lesser damage is caused to RBCs and in some way, which needs further investigation, Hb content is proportionately lesser to RBCs. Therefore MCH is also reduced. The considerably significant increase in MCH values of hydrocortisone treated and infected rats suggests that compared to insulin and thyroxine, hydrocortisone is a powerful resistance reducing agent. This is evident by drastic increase in the number of trypanosomes in hydrocortisone treated rats (Lemalatha and Narasimha Rao 1985). This point is further corroborated by rise in the colour index of the hydrocortisone treated and infected rats. The rise in the CI suggests the occurrence of hyperchromic type of anaemia which may be due to greater loss of RBC (about 76%), but lesser decrease of Hb (about 40%). In the hydrocortisone treated group, the hyperchromic type of anaemia is considerably higher compared to other two treated and infected groups.

The fall of iron content is proportional to that of Hb as the major amount of iron in the blood is associated with the haemoglobin and to some extent with β-globulins.

Thus, the considerable fall in Hb and iron contents in hydrocortisone treated and infected group suggests that hydrocortisone reduces the resistance of the hosts to infection whereas thyroxine gives protection to rats (Knowles and Das Gupta 1927).

The insignificant decrease of RBC count in treated rats and significant increase in treated and infected rats suggest that hydrocortisone is ineffective to resist the entry of trypanosomes whereas thyroxine is effective to some extent. The lowered RBC count in various groups can be attributed to the rapid multiplication of trypanosomes leading to physical damage, destruction of RBCs either mechanically or chemically due to the release of certain toxic metabolites by the parasites, absence of antibodies, weakening of the animal due to hormone treatment, and inefficient erythropoietic mechanism etc.

The enhancement of WBC count is a well known phenomenon in the infections. It is an established fact that humoral factors, a number of factors from tissue extracts rich in DNA, leucopoietin G of plasma, bacterial pyrogens and adrenocorticosteroids have been found to have leucopoietic property. The remarkable increase in the hydrocortisone treated and infected rats compared to insulin and thyroxine treated and infected rats suggests that hydrocortisone adds to pathogenicity by increasing the growth rate of trypanosomes (causing trypanosomiasis), whereas the latter gives protection to the host. The increase in the leucocyte count during parasitic infections can be justified considering the physiological significance of leucocytes such as phagocytic action, release of toxins and globulins; role in tissue repair, blood clotting etc.

The fall in the blood sugar level of infected animals is evident from the available literature. The decrease in the blood sugar may be due to its absorption by the parasites and competition between the parasite and the host for the available limited resources. The decrease of blood sugar in insulin treated animals can be attributed to the effect of insulin, in enhancing the rate of withdrawal of blood sugar in organs. In the case of other two hormones, it may possibly be due to excessive hormonal or physiological stress, leading to lowered blood glucose level. In treated and infected albino rats, both factors i.e. the competition of the trypanosomes with the host for the nutrients and the hormonal action may act cumulatively, affecting the steep fall in the blood glucose content.

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Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures

MRINAL GHOSH* and AMALESH CHOUDHURY
Department of Marine Science, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India
*Ecology Division, Zoological Survey of India, 8, Lindsay Street, Calcutta 700 087, India

MS received 2 January 1986

Abstract. Six strains of amoebae belonging to two species, viz *Acanthamoeba culbertsoni* and *Acanthamoeba rhysodes*, were isolated during the 3 prominent seasonal periods of premonsoon, monsoon and postmonsoon from the south of Sagar Island facing Bay of Bengal (21°56′ to 21°88′ N and 88°08′ to 88°16′E). The actual sampling sites were the lowest low tide belt areas. They were co-cultured in pairs, for respective seasonal periods, in 20‰ saline distilled water agar medium with their habitat associated microbiota as food. Dominance of all the seasonal isolates of *Acanthamoeba culbertsoni* over the corresponding strains of *Acanthamoeba rhysodes* arising out of competitive growth has been observed. Thus in laboratory maintained cultural conditions, strains of *Acanthamoeba culbertsoni* show remarkable adaptive superiority over those of *Acanthamoeba rhysodes*.

Keywords. *Acanthamoeba culbertsoni; Acanthamoeba rhysodes*; co-culture.

1. Introduction

Microhabitat, being the unit of protozoan ecology, may indeed be invaded by many species, but successful colonization depends on interaction with other organisms (Bamforth 1981). Stout (1952) pointed out that different species of soil protozoa occur in characteristic groupings which can be correlated with soil types. Protozoan communities are characterized by their short life history and rapid turnover which results in the availability of nutrients for recycling and the enhancement of the overall metabolic activity of the ecosystem (Stout 1974). Stout (1974) differentiated two basic patterns of organizations in most protozoan communities: one is dependent upon bacterial proliferation while the other is associated with some form of plant growth. It is now widely known that soil protozoan fauna exhibit a marked tolerance to varying environmental conditions with the ability to react quickly to favourable and unfavourable factors. Thus we see, in soils of high productivity there is the predominance of naked forms including flagellates, ciliates and naked amoebae. It is evident that rhizopods can exploit the large 'benthic' surface offered by shallow moisture films favouring interface locomotion (Bamforth 1980).

The amoeboid way of life is ecologically very successful because crawling forms can move in those shallow films; they can enter tiny spaces with their pseudopods; and they can change course merely by pushing out their body in a new direction (Bamforth 1981). Naked amoebae including 'limax' forms (e.g., *Naegleria*, *Vahlkampfia*, *Hartmannella*), *Acanthamoeba*, *Thecamoeba* and *Mayorella* are ubiquitous and usually furnish 50–90% of the protozoa in soils and litters (Bamforth 1980). Large amoebae including *Vampyrella*, *Biomyxa* and *Gephyramoeba* are also found. Additionally, genera like *Naegleria* and *Tetramitus* can transform into flagellate stages, thus exploiting swimming locomotion.

This overall dominance amongst the protozoans in soil ecosystem is paralleled by their interspecific competition. Members of the genus *Acanthamoeba* have been found as common inhabitants of the coastal mangrove fringing belts of lower deltaic Bengal (Bhattacharya *et al* 1985; Choudhury and Ghosh 1985; Ghosh and Choudhury 1985; Ghosh *et al* 1985). A detailed investigation on their quantitative abundance in mangrove litter-soils revealed the dominance of *A. culbertsoni* over *A. rhysodes* and *A. astronyxis* (Ghosh *et al* 1985). It has already been reported that the lowest low tide isolates of *Acanthamoeba* can well be grown in saline distilled water agar (SDWA) medium (Ghosh and Choudhury 1985). In the present investigation seasonal isolates of *A. culbertsoni* and *A. rhysodes* from sand-silt substrata of the lowest low tide belt were co-cultured in 20‰ SDWA medium. Couple of strains isolated during the same seasonal period but members of one species or the other, were used at one particular experimental set. The overall growth then would reflect the relative dominance, if any, of one particular strain over the other, under such situations.

2. Materials and methods

2.1 *Sampling*

Samples from 3–5 cm depth of sand-silt beds of the lowest littoral zone were collected aseptically. These sites are normally exposed during the lowest ebb of spring tide for 2–3 days per fortnight while during neap tide remain submerged to depths of about 2–3 metres. All the samples were transported to the laboratory and processed within 24 hr.

2.2 *Isolation and identification of the strains*

Seasonal samples were inoculated on to food microorganisms in respective bay water agar petri dishes. Filtered bay water served as the fluid base. Clones of *A. culbertsoni* and *A. rhysodes* strains were established according to the method of Singh and Hanumaiah (1979). Identity of the species were confirmed by reacting the homogenates with anti *A. culbertsoni* and anti *A. rhysodes* sera raised in rabbit. These isolates were maintained through subcultures, once a month to once a quarter.

2.3 *Food source*

Since amoebae are capable of using a wide range of food sources, predominantly microflora, we used their habitat associated microbiota as food. These organisms from respective samples were grown on saline adjusted bacterial nutrient agar slopes. Ingredients required were: 1 litre of glass distilled water (pH 6·4); NaCl, 20·0 g; Bacto agar (Difco Laboratories, USA), 20·0 g; Na_2HPO_4, 2·60 g; glucose, 2·0 g and Bacto tryptone (Difco Laboratories, USA), 20·0 g. The mixture was boiled in water bath, dispensed suitably and autoclaved.

A little quantity of samples were inoculated aseptically on agar slopes and kept overnight at 37°C incubator. To avoid unwanted particles etc., they were subcultured several times. These mixed cultures of food microbiota were used for isolation and subsequent maintenance of respective clonal isolates.

2.4 *The culture media*

2.4.1. *Isolating medium*: One litre of filtered seasonal bay water from the littoral zone and Bacto agar 15·0 g were boiled and autoclaved.

2.4.2. *Experimental medium*: One litre of glass distilled water; NaCl 20·0 g and Bacto agar 15·0 g pH was adjusted to 8·25 which is the approximate mean value of seasonal sampling water. These mixtures were boiled, autoclaved and dispensed in sterile 7·5 cm dia petri dishes. Media plates were suitably dried at room temperature before use.

2.5 *Modified Page's amoeba saline*

The modified Page's amoeba saline (PAS) (Page 1966) was made as follows: 1 litre of glass distilled water; NaCl, 20·0 g; $MgSO_4 \cdot 7H_2O$, 0·004 g; $CaCl_2 \cdot 2H_2O$, 0·004 g; Na_2HPO_4, 0·142 g and KH_2PO_4, 0·136 g.

2.6 *Experimental*

Laboratory maintained young cultures of microbiota were used as food. Final wet weight suspensions of 0·05 g/ml were made in 3 tubes for each of the 3 experimental sets for 3 seasonal isolates in modified PAS. Five replicate plates for each pair of competitive strains for 3 seasonal periods were spread with 0·2 ml of the suspending fluid so that each gets about 0·01 g wet weight of microorganisms. When the excess fluid was absorbed, cysts in pairs, for respective isolates were inoculated on media plates. The PAS medium was allowed to absorb and the plates were inverted and kept at 25 ± 1°C BOD incubator. Data for the overall population growth after 100 days of culture were taken and the values were expressed in counts/petri dish (mean of 3 replicate plates). Both the co-culturing strains as well as those growing separately on parallel cultures were considered. It is always probable to lose some of the organisms while harvesting.

3. Results

The growth of all the competitive sets of strains for all the 3 seasonal periods have been documented. The same of parallel cultures of seasonal isolates of *A. culbertsoni* and *A. rhysodes* have also been plotted. Overall growth of the experimental strains after 100 days of culture are plotted in figure 1. Maximum growth were encountered for monsoonal isolates in both the species being followed by postmonsoon and premonsoon growth in sequence. This bar diagram also depicts the dominance of all the strains of *A. culbertsoni* over the corresponding strains of *A. rhysodes*.

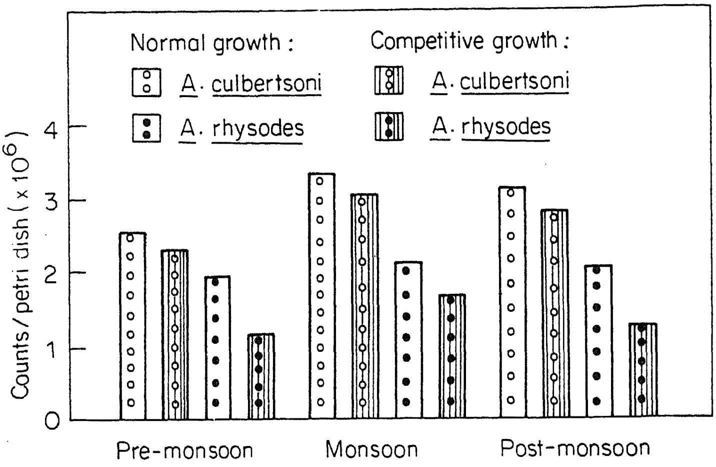

Figure 1. Growth of *A. culbertsoni* and *A. rhysodes* strains under co-cultural condition in 20‰ SDWA medium.

4. Discussion

Dominance is an expression of ecologic inequalities, arising out of different exploitations of the environment (McNaughton and Wolf 1970). Protozoa constitute a relatively small component in ecosystems with a large biomass, while in ecosystems with much smaller biomass they may constitute a much higher proportions. In ecosystems with fluctuating biomass they exhibit concomitant changes (Stout 1974). Competition can cause niche differentiation while small changes in the activity of microbial populations in soil can affect significant changes in soil metabolism (Stout 1973). The relative abundance of the 3 species of *Acanthamoeba*, viz *A. culbertsoni*, *A. rhysodes* and *A. astronyxis*, from mangrove litter-soils, both in the active and inactive stages has already been investigated (Ghosh *et al* 1985). In the present study, under cultural condition, the dominance of the strains of *A. culbertsoni* over those of *A. rhysodes* is clearly evident. This dominance however, might result from the presence of more amount of food amongst the supplied heterogeneous microbiota preferred by *A. culbertsoni* strains in relation to those of the competitive strains, or that they could have interacted with microbiota in a better way or else, they could inhibit the growth of *A. rhysodes* strains by producing some excretory and/or secretory factors in the culture medium while themselves being less affected by those produced by the competitive strains.

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Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city with a note on mite infestation

B AYYACHAMY DANIEL, CHERIYAN THOMAS,
K KALYANARAMAN* and R S PRASAD

Department of Zoology and *Department of Statistics, University of Kerala, Kariyavattom,
Trivandrum 695 581, India

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Abstract. A study relating to seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city was carried out between August 1984 and July 1985. The area chosen was the one which is a perennial breeding source for *Mansonia* mosquitoes. Only *Mansonia uniformis* and *Mansonia annulifera* were prevalent in this area of which the former was found to be the predominant species. Decrease in the number of *Mansonia annulifera* from the survey conducted during the previous year may be due to the disappearance of *Pistia stratiotes* and its replacement by *Salvinia auriculata*. Rainfall and temperature are the two important environmental factors which influence the number of *Mansonia* mosquitoes visiting the host. The maximum number of adults of *Mansonia uniformis* was collected during February to June 1985. No swarm of *Mansonia uniformis* was encountered during the present study, however, males were found to wait and mate with the resting as well as blood fed females in the vicinity of the host. Both sexes of *Mansonia* mosquitoes were parasitized by *Arrenurus* and limnesiid species of water mites. Maximum parasitization was seen during July 1985.

Keywords. *Mansonia*: seasonal prevalence; host visitation; parasitization.

1. Introduction

Much emphasis has been laid on the problem of filariasis (both wuchererian and brugian) in India during the past few decades (Buck 1976; Sharma 1976; Das 1976). *Brugia malayi* filariasis is limited to small pockets in peninsular India and less common in North West (Chandrasekharan *et al* 1976). Three species of *Mansonia* mosquitoes viz *Mansonia annulifera*, *Ma. uniformis* and *Ma. indiana*, play an important role in the transmission of this disease, of which *Ma. annulifera* is the most important vector in Kerala state (Nair 1962; Nair and Roy 1958; Jaswant Singh *et al* 1956; Chandrasekharan *et al* 1976).

Taking into consideration the vectorial status of mosquitoes and in the absence of any previous entomological survey after 1938 relating to prevalence, seasonal fluctuations and host visitation, a study was undertaken in Trivandrum city. Trivandrum is a fast developing city and several marshy, water-logged areas suitable for the growth of *Pistia stratiotes*, *Salvinia auriculata, Eichhornia speciosa* etc., are being cleared off and filled up for building constructions. Ecologically the most fascinating aspect of Trivandrum is that though the city is being converted into a 'concrete jungle' with massive building construction, yet it is possible to see open drainage canals and paddy fields stocked with water plants.

2. Materials and methods

2.1 *The survey area*

An extensive survey of the breeding areas of *Mansonia* mosquitoes in Trivandrum district (total population 2,591,057 and population density 1,182/sq km) with special reference to areas in and around Trivandrum city (total population 4,82,722 and population density 6,442/sq km) was carried out. Though *Mansonia* mosquitoes were found breeding in 5 areas within the city limit (figure 1), the present study was concentrated on one of these areas called Kannanmoola. The study area has a canal

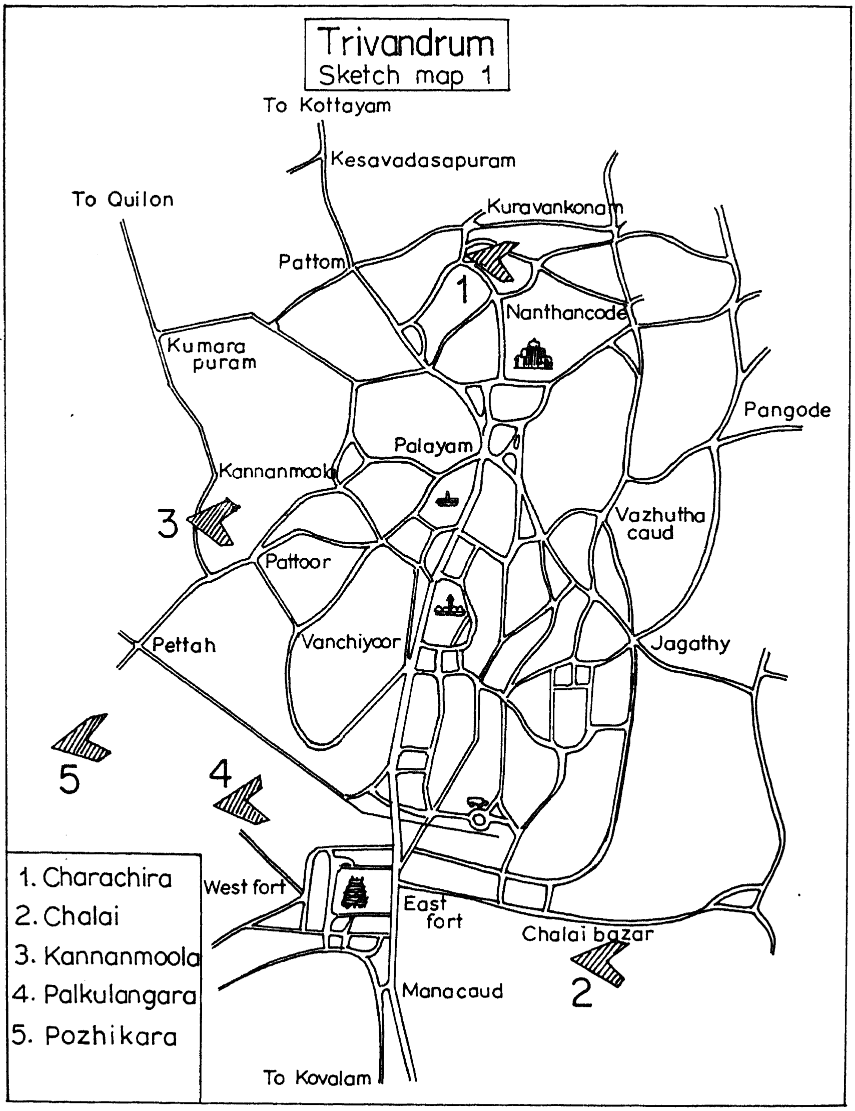

Figure 1. Map of Trivandrum city. Arrows show different collection spots of *Mansonia* mosquitoes.

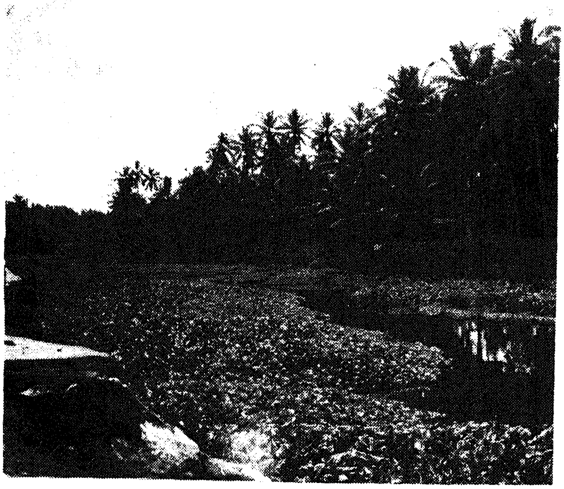

Figure 2. Running water in the middle and intense growth of *Eichhornia speciosa* on either sides of the drainage canal in Kannanmoola in Trivandrum (× 750).

which runs through the city into which small canals from different parts of the city drain. The major canal empties into a lake about 3 km away from Kannanmoola. The canal is 3 m deep, characterised by running water in the middle and intensive growth of *E. speciosa* as the permanent aquatic plant on its either sides (figure 2). The study was started with areas where *Pistia* plants predominated, but are now being replaced by *S. auriculata*.

2.2 *Meteorological conditions*

Necessary data relating to temperature, rainfall, humidity and wind velocity were procured from the Central Meteorological Department, Trivandrum, which is located about 5 km away from the study area.

2.3 *Adult mosquito collection*

The adults of *Mansonia* mosquitoes were collected from a cattle shed located very near to the canal in Kannanmoola, twice a week between 18·45 and 19·30 hr. The cattle shed was illuminated with a 60 watt bulb and the mosquitoes were hand collected using test tubes (WHO 1975). A flash light was used during the collection. The number of mosquitoes per man hr for each collection was calculated. The field-collected mosquitoes were brought to the laboratory, identified, examined for mite infestation and the presence of fresh blood in the gut. The parasitic mites infested on the adult mosquitoes were sent to Dr G R Mullen, Auburn University, USA, for identification.

2.4 *Study on swarming mosquitoes*

In order to study the swarming behaviour of *Mansonia* mosquitoes in and around the survey area, swarming insects were collected by hand net as described by WHO (1975).

Collections were made during 18·30–19·00 hr. Insects thus collected were brought to the laboratory and identified.

2.5 *Statistical methods*

In order to bring out any possible correlation between physical factors like temperature, rainfall, wind velocity and the fluctuations in the host visiting populations of *Mansonia* mosquitoes, statistical methods like Partial correlation and Student's '*t*' test were used.

3. **Results**

3.1 *Adult mosquito collection*

Of the total of 8,113 mosquitoes collected during the period of the study, *Mansonia* species constituted 65·32% (5301). Of these, 97·66% were *Ma. uniformis* and only 2·34% were *Ma. annulifera.* Other mosquitoes species collected during the study are shown in table 1.

3.2 *Seasonal fluctuation of Ma. uniformis in the whole year*

Figure 3 depicts *Mansonia* mosquitoes collected, in relation to rainfall and temperature during the one year period of study. Though *Ma. uniformis* was represented in the year-round collection, the number of mosquitoes visiting the host during the study period varied from 1–194 mosquitoes/man hr. The maximum number of *Ma. uniformis* was collected in February 1985 (118 mosquitoes/man hr) and minimum in October 1984, (35 mosquitoes/man hr). One of the characteristic features of the collection was the presence of males of *Ma. uniformis* in fairly large numbers in the cattle shed whereas males of no other mosquitoes were found in the shed.

Table 1. Other mosquito species collected during August 1984 and July 1985.

Name of species	Frequency	Peak period
Culex gelidus	Very common	Nov.–March
Cx. sitiens	Very common	Nov.–March
Anopheles hyrcanus	Common	June–August
Aedes vexens	Less common	—
Ae. vittatus	Less common	—
Armigeres subalbatus	Less common	—
Ficalbia chamberlaini	Less common	—
An. jamesi	Rare	—
An. vagus	Rare	—
An. barbirostris	Rare	—

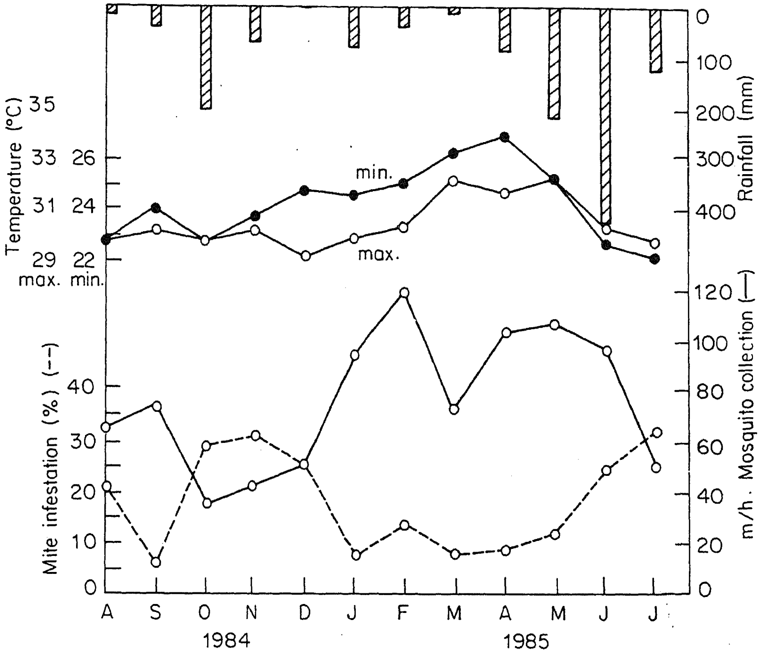

Figure 3. Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Kannanmoola, Trivandrum city, in relation to rainfall, temperature (minimum and maximum) and percentage of mite infestation.

3.3 *Influence of climatic factors on the prevalence of Mansonia mosquitoes*

The rainy season was between April–July and October–November, during the period of study. The monthly mean maximum temperature during the hottest month (April) went up to 33·9°C and the monthly mean temperature in the coldest month (December) went down to 22·1°C. Wind velocity was maximum during June and minimum in December. As it is evident from figure 3, there was a steep rise in the number of mosquitoes visiting the host from October–February. This obviously was a peak period of collection of mosquitoes (35–118 mosquitoes/man hr). In the subsequent months though there was a slight fall in the number of mosquitoes it never reached as low as that of October. But during April–May the number remained high. There is a sudden fall during June–July month. Statistical analysis has shown that minimum temperature and rainfall are the two environmental factors which show correlation with the flight activity of the mosquitoes. As it is evident from table 2, minimum temperature and rainfall have correlation coefficient of 0·34 and 0·76, respectively with the number of adult mosquitoes. Both are significant at 5% level with a *t*-value of 3·27 and 2·44, respectively. It can be surmised from figure 3 as well as the statistical calculations that the highest activity of mosquitoes appears to fall during the period when the minimum temperature falls within the range of 23–25°C.

The influence of the minimum temperature is something which can easily be understood as the collections were made at dusk. However high the day time temperature may be, it could not affect the mosquitoes because this constitutes the resting phase in their daily activity rhythm.

Table 2. Showing correlation matrix between total adults collected, temperature, rainfall and wind velocity.

	A	$T_{\max}$	$T_{\min}$	R_f	W_v
A	1	0·026	0·34[a]	0·26[a]	−0·11
$T_{\max}$		1	0·48	0·38	−0·37
$T_{\min}$			1	0·76	−0·64
R_f				1	−0·47
W_v					1

[a]Significant at 5% level.

A, Adult mosquitoes; $T_{\max}$, temperature maximum; $T_{\min}$, temperature minimum; R_f, rainfall; W_v, wind velocity.

A sudden decrease in the number of adults visiting the host during June–July appear to have some correlation with the onset of rain. There appears to be a decline in the number of mosquitoes visiting the host during onset of the monsoon months. Kerala gets two monsoon periods, the North–East in October and November and South–West in July and August. These are the two periods when a sharp decline in the number of mosquitoes visiting the host is observed.

3.4 *Swarming and mating*

The swarm encountered in the study area consisted almost entirely of *Culex gelidus*. It is of great importance to notice that no *Mansonia* mosquito was collected during any time of the year from the mosquito swarm examined. It should also be noted that only males of *Ma. uniformis* were collected from the cattle shed from where the routine collection was made. It was also observed from the hand net collection that it was the male *Ma. uniformis* which arrived first to the cattle shed followed by the females.

3.5 *Mite infestation*

Of the 5,182 *Ma. uniformis* collected 20·78% were found to be parasitized by water mites. The incidence of parasitization of *Ma. uniformis* male mosquito was 24·52% whereas that of female was found to be only 14·37%. Of the 121 female *Ma. annulifera* collected only 18 (14·88%) were infested by water mites. Since, only the larval stages of mites associated with the adult mosquitoes were encountered during the present study, their determination at species level was found to be difficult. However, based on larval features alone, they were determined to be two species of *Arrenurus* (family—Arrenuridae) which were tentatively designated *Arrenurus* species A-B, and a limnesiid (family—Limnesiidae) by Mullen G R (unpublished results). Of the total *Arrenurus* infested mosquitoes, collected from the study area, 51% were found to be attached to the cervical region, 48% to the abdominal region and 1% to the other regions like head, proboscis, leg and wing. With regard to limnesiids, they were primarily attached to the cervical region, accounting for 96·6% of the attachments. Only a few were attached to the abdominal venter and wing. Simultaneous infestation by both species of *Arrenurus* and limnesiids on the adult *Mansonia* mosquitoes is a common feature observed in the present study. *Arrenurus* species preferred to be attached on the abdominal venter and that of limnesiids on the cervical region.

The graph relating to abundance of infestation shows a typical bimodal one, but in the reverse pattern. There appears to be a reverse relation between the rate of mite infestation and the total number of mosquitoes caught. Maximum infestation of mite on the adult mosquitoes appear to be from October–December 1984, and June–August 1985, the periods when the number of *Mansonia* mosquitoes collected was minimum.

4. Discussion

It appears that the entomological survey of *Mansonia* mosquitoes carried out in Trivandrum city during the course of the study is the first of its kind after Iyengar (1938). It is a well known fact that *Ma. uniformis* is the principal potential vector of *B. malayi* infection. Nevertheless, the existing records show that at present Trivandrum is free from *B. malayi* infection. However, *B. malayi* infection is still present in small pockets of Alleppey district in Kerala. As there is constant movement of people between these two places, the possibility of introduction of *B. malayi* infection into Trivandrum city cannot be ruled out.

Earlier work carried out so far, in Kerala has shown that the peak season for *Ma. annulifera* was from January–June (pre-monsoon species) and that for *Ma. uniformis* was from July–November (post-monsoon species). The most favourable period for *Ma. annulifera* appears to be the most unfavourable period for *Ma. uniformis* and *vice versa* (Chandrasekharan *et al* 1976). The factors which regulate 'favourableness' and 'unfavourableness' are not known. However, in the present study, the 'favourable' or 'unfavourable' phenomena exhibited by these two species of *Mansonia* mosquitoes have not yet been encountered during the one year period of study. Moreover, a perusal of the annual trend of density of these *Mansonia* mosquitoes during host visitation, in 1984–85, showed a declining trend for *Ma. annulifera* and highly fluctuating trend for *Ma. uniformis*. Earlier studies carried out during 1931–34 by Iyengar (1938) and in 1956 by Jaswant Singh *et al* (1956) showed that in Trivandrum, *Ma. annulifera* was the most predominant species among *Mansonia* mosquitoes. During those periods *Ma. annulifera* bred in abundance on roots of *P. stratiotes*, commonly observed in tanks, canals and rivers of Kerala State (Burton 1959). Subsequently, an introduced water fern, *S. auriculata*, during 1960, crowded out *Pistia* and formed an impervious growth that increased larval mortality (Joseph *et al* 1963), which in turn, will naturally reflect upon the declining trend of *Ma. annulifera* population.

Major ecological change noticed in *Mansonia* breeding areas in Trivandrum city, during the present study is the disappearance of *P. stratiotes* which is now replaced by *S. auriculata*. Another noteworthy feature which need mention here is that, during 1983, of the total adult *Mansonia* mosquitoes emerged in the laboratory, *Ma. annulifera* constituted 55·3% from the field collected larvae, whereas, it was only 9·88% in 1984. Moreover, in 1983, when the survey of *Mansonia* larvae were conducted in Kannanmoola area, the *Pistia* plants were present as thick beds which gave harbourage for the imaginal stages of *Mansonia* mosquitoes. Unfortunately, during the survey in 1984, the *Pistia* plants had almost disappeared from the canals leaving only small patches of *Pistia* in between the *S. auriculata* or *E. speciosa*. An possible correlation between the decline in the population of *Ma. annulifera* and th disappearance of *Pistia* plants needs further study. However, it may be noticed tha *Ma. uniformis* was unaffected by these changes. They appear to have adapte themselves to other aquatic plants especially *Eichhornia*. The laboratory studies hav also shown that *Ma. uniformis* can well thrive on other aquatic plants lik *S. auriculata, E. speciosa, Ipomoea aquatica, Commelina nudiflora* etc.

Very little information is available on mites parasitizing mosquitoes in India. O the known 11 acarine families containing species known to parasitize or prey o mosquitoes (Mullen 1975a; Smith 1983), only two representatives from the familie of prostigmatid water mites (family Arrenuridae and Limnesiidae) were reported i the present study.

The infestation of *Arrenurus* mite was reported on various species of mosquitoes like *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Ficalbia*, *Hodgesia*, *Mansonia* an *Uranotaenia* (Smith 1983). In Asia *Arrenurus* species have been known to parasitiz a total of 12 species of *Anopheles*, *Culex* and *Mansonia* (Reisen and Mullen 1978) Of the various species of mosquitoes collected during the present study onl *An. hyrcanus*, *Ma. annulifera* and *Ma. uniformis* have been found to be parasitize by *Arrenurus* species A and B. However, no other mosquito collected during th present investigation were found to be parasitized by these water mites.

Limnesiid mite larvae parasitizing *Mansonia* mosquitoes reported in the presen study is the first of its kind in India as the earlier workers have observed onl *Arrenurus* species parasitizing mosquitoes (Saxena and Sharma 1981; Rahman *et a* 1979; Malhotra *et al* 1983). Insofar as is known, limnesiid larvae have been reporte from a number of *Mansonia* species in Uganda and Indonesia (Mullen 1975a; Smit 1983). During the present investigation, limnesiid larvae were found to have a clea host preference for *Ma. uniformis* readily parasitizing both sexes. Moreover, most o these water mites were preferred attached to the cervical region of these mosquitoes although occasionally specimens were found on the abdominal venter and wing.

Since Gillett (1957) first proposed that the presence of parasitic water mites o female mosquitoes can serve as an indicator of host's nulliparity, considerable worl has appeared in the literature regarding the topic. More recently, Service (1973) an Mullen (1975b) supported Gillett's view. The present study is also in conformit with the findings of Gillett (1957) i.e., 80% of the total mite infested mosquitoe examined so far, for physiological age determination, were found to be nulliparous

When the abundance of parasitic mites collected during the study period wa compared, the mean parasitic load was higher for *Ma. uniformis* females than for th males. One should remember that with regard to *An. hyrcanus*, no male wa collected from the cattle shed (some of the females of *An. hyrcanus* were infeste with water mites). A bias for female hosts would be advantageous because mal mosquitoes are much less likely to return to water after emergence (Stechman 1980 Smith and McIver 1984). The apparent sex bias is due to male abundance (mal emergence precedes female emergence) correspondingly closer to female emergenc rather than the mites discriminating between sexes (Smith and McIver 1984).

In the present study, mite infestation on adult *Mansonia* mosquitoes wa maximum from October–December 1984 and June–August 1985, during which th number of mosquitoes collected was minimum. In spite of the fact that some of th mosquitoes are heavily parasitized by these water mites, the mosquitoes fly from their breeding source to the host for their blood meal. However, the heavy infestation of these water mites influencing longevity, flight activity and reproduction are yet to be studied in detail.

Two important physical factors which appear to influence the number of mosquitoes visiting the host during the one year study were temperature and rainfall. The minimum temperature has the best correlation with the host visiting abundance of *Mansonia* mosquitoes, whereas the maximum did not show statistical significance. Earlier workers like Hayes and Hsi (1975) and Hayes and Downes (1980) reported a correlation with the temperature and population abundance with *Culex pipiens quinquefasciatus* and indicated that temperature dependence is interwoven throughout all stages of the mosquitoes' life cycle. In the present study, when the temperature is high the *Mansonia* mosquitoes visiting the host were found to be high.

It is possible that rainfall also has some indirect influence on the number of mosquitoes visiting the host. The finding reported here clearly documents the correlation of host visitation abundance of *Mansonia* mosquitoes with rainfall in Kannanmoola. There appears to be a sharp decline in the number of mosquitoes visiting the host during the onset of monsoon periods. The correlation for *Mansonia* mosquitoes using partial correlation and Student's '*t*' test suggest that very large increase in the rainfall may not be accompanied by proportionately large increase in the number of the mosquitoes visiting the host. These findings are consistent with those of Reisen *et al* (1977) and Olson *et al* (1983) reported for *Culex* mosquitoes. The close correlation and relative abundance of *Mansonia* mosquitoes visiting the host with rainfall provides an extremely useful index for prediction of success of hand collection of these mosquitoes based on data readily available from almost any *Mansonia* breeding area.

The total rainfall was high during April–July 1985. Correspondingly an increased number of *Ma. uniformis* was observed during the same period. In contrast, during October 1984, though the rainfall was 215·1 mm the number of host visiting mosquitoes was less. It is possible that due to sudden flooding in October (North East monsoon period) the canals were flooded with water and thus the velocity of the running water was increased. Owing to the flooding and strong water currents in the canals, aquatic plants like *Eichhornia*, *Salvinia*, *Pistia* etc., were carried from their original place to a distant area. Usually, these aquatic plants, especially *E. speciosa*, will have their roots buried in the top layer of the loose silt in water. One should bear in mind that, the imaginal stages of *Mansonia* mosquitoes are attached to the roots of these aquatic plants for their various physiological processes in their breeding grounds. Thus due to flooding and strong water currents, the aquatic plants along with the imaginal stages of the mosquitoes were uprooted and carried to a far off place from the original breeding site. Moreover, after emergence the mosquitoes from these areas may find it difficult to reach the original breeding grounds and visit the host for their blood meal. Thus, obviously, there was a decrease in the number of mosquitoes visiting the host in the study area. This holds true in the present study, during October and November 1984.

The present study clearly brings out the fact that there was no swarm of *Ma. uniformis* as observed in other species of *Mansonia* (Jayawickreme 1953—cited by Mattingly 1957; Neilson 1964). Swarming and mating behaviour of *Mansonia* mosquitoes were studied earlier by Shute (1930), Jayawickreme (1953—cited by Mattingly 1957) and Neilson (1964). During swarming, the males of *Ma. uniformis* wait in a zone which is likely to be traversed by females as in the case of some Simuliids where the males swarm over cattle and catch the female as they come to feed (Wenk 1965).

It appears that males locate the host and arrive at the site early and wait for the females to come. Males were found to mate with resting as well as blood fed females in the vicinity of the cattle. Field experiments conducted by McIver (1980) and McIver *et al* (1980) demonstrated that male *Mansonia* mosquitoes were attracted to hosts from at least 3 m and their density was found to be significantly greater than at a distance of 7 m from the bait for the females. The sensory stimulus used by *Mansonia* males in orientation are in all probability, olfactory cues contained in the odour plume emanating from the host (McIver 1980). The use of olfactory cues for long range orientation as reported by McIver (1980) is probably the most important mechanism operative in *Ma. uniformis*. This kind of behaviour might have facilitated in the present investigation for the mate-finding phenomenon, to bring the sexes together in the vicinity of the host.

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Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records

KENNETH A SPENCER
Department of Biological Sciences, University of Exeter, Exeter, EX4 4QJ, England

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Abstract. Six new species are described: *Melanagromyza erawanensis* sp.nov., *Melanagromyza inthanonensis* sp.nov., *Melanagromyza pasiae* sp.nov., and *Melanagromyza pasiensis* sp.nov.; *Tropicomyia pilosa* sp.nov.; and *Pseudonapomyza rampae* sp.nov. Two new synonyms are established in the genus *Cerodontha*, subg. *Icteromyza-hirsuta* Sasakawa with *piliseta* (Becker) and *hardyi* Sasakawa with *duplicata* Spencer; *Phytagromyza santokhi* Garg from India is synonymised with *Pseudonapomyza atrata* (Malloch). *Cerodontha rishii* Garg from India is resurrected from synonymy. Fourteen species are now recorded as new to Thailand, making the total now to 27.

Keywords. Agromyzidae; male genitalia; aedeagus; leaf mine.

1. Introduction

Agromyzidae have hitherto been poorly known in Thailand, with no specialised collecting having been undertaken, apart from a few days I spent in Bangkok in 1960. The forested mountains in the north generally west of Chiang Mai represent the eastern outliers of the Himalaya and are an area of great faunal interest, contiguous with the mountains of SW China and with unimpeded access to the south.

The primary object of this paper is to record the Agromyzidae discovered during my expedition to Thailand from 15 November to 5 December, 1985. I spent only a single day in Bangkok, collecting in the large Ja-tu-jak Park opposite the Central Plaza (which is now unfortunately largely occupied by a golf course) and for the remainder of the time I was based at Chiang Mai, 700 km to the north, collecting mainly in forests in mountainous areas up to the summit of Doi Inthanon, 2590 m. I also flew to Mae Hong Son, 130 km NW of Chiang Mai and from there travelled north-west into the mountains to within 20 km of the Burma border.

Identification of the material collected has necessitated some revision of Oriental species, with examination of types of *Agromyza panici* de Meijere from Java and *Pseudonapomyza alternantherae* (Séguy) from Madagascar, and the male genitalia of the types of both species are illustrated for the first time. The discovery of 6 new species in the genera *Melanagromyza*, *Tropicomyia* and *Pseudonapomyza* was not surprising but more interesting was the identification of species which greatly extended their known range, such as *Agromyza panici* de Meij. only known from Java, *Ophiomyia rotata* (Spencer), previously only known in the Philippines and Australia, *Cerodontha* (*Cerodontha*) *ipei* Garg, known only from India, *Phytoliriomyza australensis* Spencer, described from Australia and later recorded in Nepal, and finally *Phytoliriomyza tricolor* (Malloch), which has hitherto been accepted as endemic in Australia. Following the discovery of a male of

Pseudonapomyza alternantherae (Séguy) at Chiang Mai, which was described from Madagascar and subsequently recorded in India, South Africa and Cameroun, I examined the genitalia from all these localities, including the holotype from Madagascar and the illustrations given below and earlier clearly indicate the incipient speciation which is taking place in the widely separated populations where this species is now known (see below).

With only 27 species known in Thailand out of a total of approximately 250 confirmed species in the Oriental Region, it is clear that many further species await discovery and it is hoped that this paper will encourage further collecting in Thailand of this interesting and economically important family.

The following abbreviations have been used:

ors–upper orbital bristle(s)
ori–lower orbital bristle(s)

2. Discussion of species

2.1 *Genus Agromyza Fallén*

This predominantly north-temperate genus is nevertheless well represented in the Oriental Region, particularly in the north in the Himalayas and Taiwan but no species have hitherto been known in Thailand. *A. panici*, previously only known in Java, can now be recorded from the mountains NW of Chiang Mai.

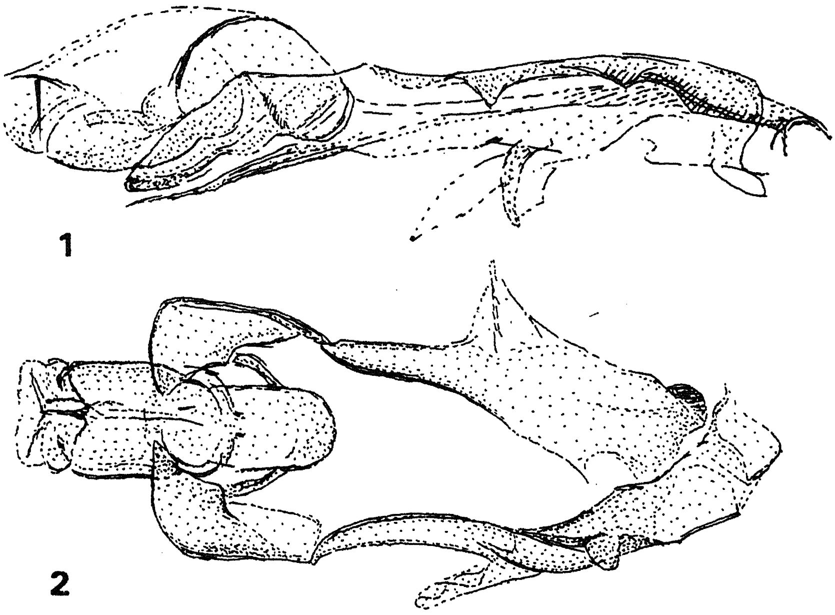
2.1a *Agromyza panici de Meijere* (*figures* 1, 2)

N. Thailand: Forestry Station, 30 km NW Mae Hong Son, 1000 m, 1 ♂, 28.xi.85.

I have examined the 3 syntypes of *A. panici*, bred from leaf mines on *Panicum palmifolium* Poir. in Java at 800 m. Of these, the male is herewith designated as lectotype and the 2 females as paralectotypes. They are mounted dry ex alcohol and in all specimens both head and mesonotum are somewhat deformed. Essential characters of this species are: largely black, wing length in both sexes 2–2·1 mm; frons narrow, equal to width of eye, dark, brownish-black, orbits shining black; orbital bristles variable, 2 upper and 1–3 lower, normally 2, orbital setulae sparse, reclinate; jowls angular at rear, $\frac{1}{4}$ height of eye; third antennal segment small, round, largely black, narrowly brownish at base; mesonotum shining black, 2 strong dorso-centrals, with a smaller 3rd which is closer to the 2nd than to the suture, acrostichals in about 6 rows; wing with costa extending to vein M1+2, inner cross-vein near centre of discal cell, last section of M3+4 approximately 2/3 penultimate section; legs: femora black, tibiae and tarsi only slightly paler, brownish-black; squamae pale, whitish-gray, with margin scarcely differentiated, fringe blackish; halteres white; male genitalia: characteristic of grass-feeders, as in figures 1, 2; sperm pump apparently minute (not found in either male examined).

De Meijere (1934: 248) stated that the mesonotum bears only 2 dorso-centrals. This would be unusual in an *Agromyza* and the small third is certainly not detectable in the syntypes owing to their poor condition. It is, however, clearly present in the specimen from Thailand.

I caught a female at Mae Glarn Waterfall, Doi Inthanon, SW Chiang Mai, 360 m,



Figures 1–2. *Agromyza panici* de Meij. 1. Aedeagus, side view. 2. Ventral view.

21.xi.85, which agrees closely with *A. panici* but the third antennal segment appears to be larger and the squamal margin is deep black. This may well represent a further species but its status cannot be clarified until a male from the same, readily accessible locality becomes available.

2.2 *Genus Melanagromyza Hendel*

Four new species are described below and new records are given of two widespread oriental species, *M. albisquama* (Mall.) and *M. metallica* (Thom.) which have not previously been recorded in Thailand.

Two other species are known in Thailand—*M. obtusa* (Mall.) (=*M. weberi* de Meij.) which feeds in pods of leguminous crops, particularly *Cajanus*, *Moghania* and *Phaseolus*, and is a pest of some significance in India (cf. Spencer 1966b, 1973: 46), and *M. sojae* (Zehntner), an internal stem-borer in many leguminous genera, including *Glycine*, *Moghania* and *Phaseolus* but is only considered as a minor pest (Spencer, 1973: 52).

The 6 species described and discussed below all belong to the large group of greenish species present throughout the Old World tropics. The eye in the male is bare in 3—*M. albisquama*, *M. erawanensis* sp.nov. and *M. pasiensis* sp.nov. but characteristically pilose in *M. inthanonensis* sp.nov., *M. metallica* and *M. pasiae* sp.nov. *M. inthanonensis* and *M. pasiae* can be further distinguished by their larger size. However, in this large and difficult group of species a positive identification of caught specimens will frequently only be possible from the male genitalia.

2.2a *Melanagromyza albisquama* (*Malloch*) (*figures* 3, 4)

N. Thailand: Doi Inthanon, 1 ♂, 3.xii.85, roadside, 1760 m; Doi Suthep, W Chiang Mai, 1,000 m, 1 ♂, 2.xii.85; 1 ♂, 2 ♀, 4.xii.85.

This species is widespread from Fiji (type locality) and N Australia to India and Africa. The only recorded host is *Desmodium frutescens* Walp., the larvae feeding in the pods. Forty-two species of *Desmodium* are recorded in Thailand (Smitinand 1980) but it seems probable that the species may feed in other genera of Papilionaceae.

M. albisquama belongs to the group of small, green species with the eye in the male virtually bare. It can only be distinguished by the male genitalia (figures 3, 4) from *M. erawanensis* sp.nov. and *M. pasiensis* sp.nov. described below. Wing length of the specimens now recorded ranges from 1·9 mm in the male to 2·3 mm in the female.

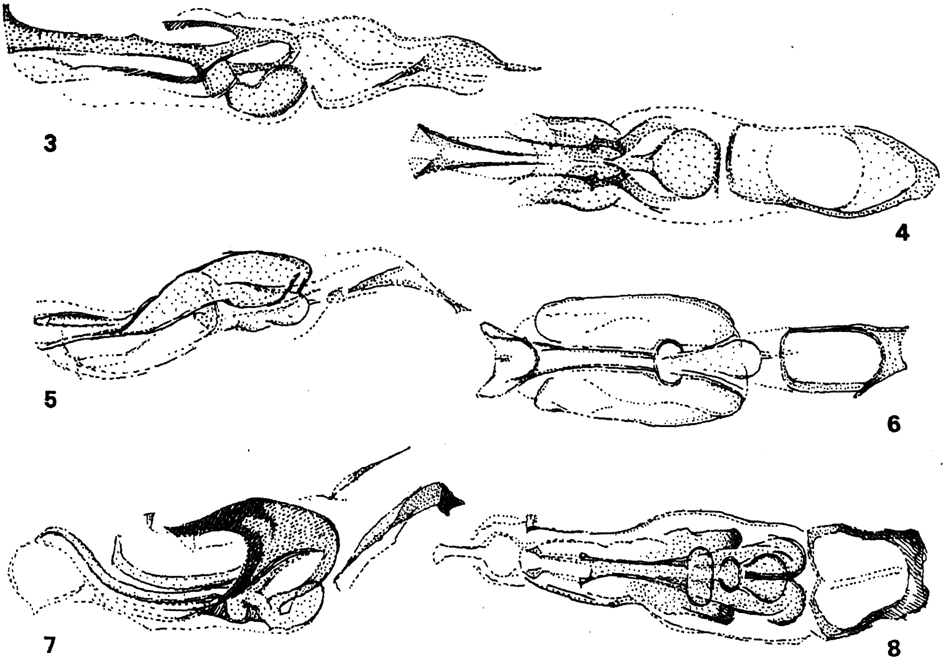
2.2b *Melanagromyza erawanensis* sp.nov. (*figures* 5, 6)

Adult: small greenish species, similar in size to *M. metallica* but differing in the virtually bare eye in the male; wing length in holotype 2·1 mm; indistinguishable on external characters from *M. albisquama* and *M. pasiensis* sp.nov.

Male genitalia: aedeagus (figures 5, 6) distinctive.

Holotype ♂, N Thailand: Erawan Resort, 20 km N Chiang Mai, 750 m, 23.xi.85, to be deposited in British Museum (Nat. Hist.).

Remarks: Although no apparent differences between this species and *M. albisquama* and *M. pasiensis* sp.nov. are detectable, the male genitalia are entirely distinct.



Figures 3–8. 3–4. *Melanagromyza albisquama* (Mall.); 3. Aedeagus, side view. 4. Ventral view. 5–6. *M. erawanensis* sp.n. 5. Aedeagus, side view. 6. Ventral view. 7–8. *M. inthanonensis* sp.n. 7. Aedeagus, side view. 8. Ventral view.

2.2c *Melanagromyza inthanonensis sp.nov.* (*figures* 7, 8)

Closely resembling *M. pasiae* sp.nov., with following slight differences: frons narrower, equal to width of eye; jowls narrower, 1/12 height of eye; wing length 2·5 mm in male, 2·6 mm in female; male genitalia: aedeagus (figures 7, 8) complex, distinctive.

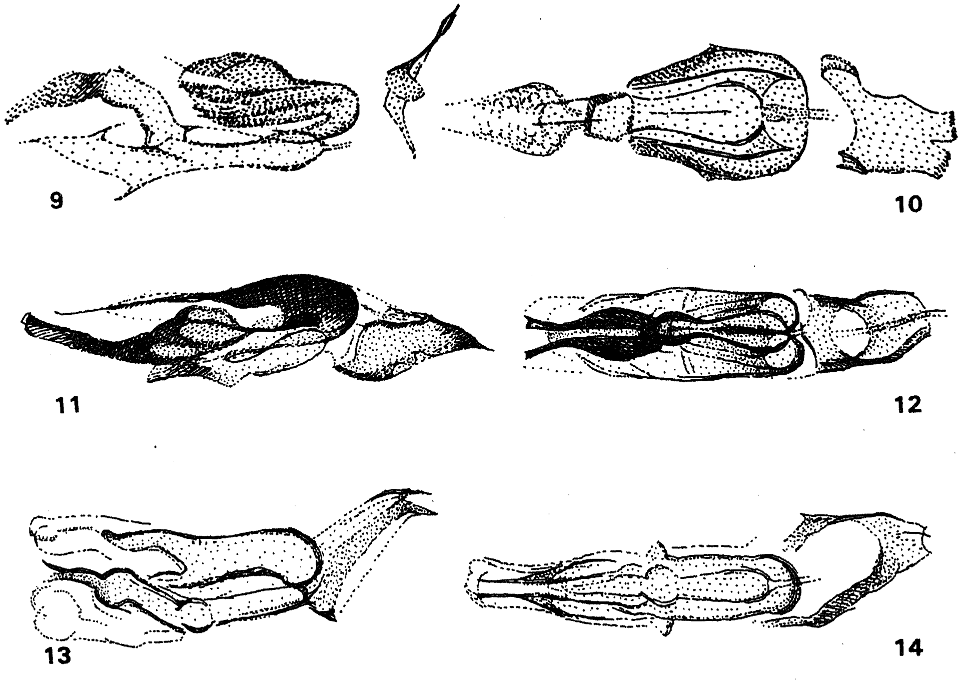
Holotype ♂, N Thailand: Doi Inthanon, 1,400 m, 20.xi.85, to be deposited in British Museum (Nat. Hist.). One female, same locality and date, has similar narrow jowls but the frons is broader, 1½ times the eye height and this specimen is not treated as a paratype.

Remarks: The male genitalia indicate relationship with *M. cordiophoeta* Spencer (1961a: figure 43) described from Singapore. Sasakawa (1963b: figure 6) also illustrated the genitalia of a specimen from Papua New Guinea. However, in *M. cordiophoeta* the eye in the male is bare.

2.2d *Melanagromyza metallica* (*Thomson*) (*figures* 9, 10)

N Thailand: 22 specimens, 22.xi.–6.xii.85, Chiang Mai, Doi Suthep, Mae Sa Botanical Gardens and Erawan Resort.

This is probably the most common species in the Old World tropics, with its range extending from northern Australia and Papua New Guinea to India and Africa but it



Figures 9–14. 9–10. *M. metallica* (Th.). **9.** Aedeagus, side view. **10.** Ventral view. **11–12.** *M. pasiae* sp.n. **11.** Aedeagus, side view. **12.** Ventral view. **13 and 14.** *M. pasiensis* sp.n. **13.** Aedeagus, side view. **14.** Ventral view.

has not previously been recorded in Thailand. It belongs to the complex of small greenish species, with the male having a conspicuous patch of white pilosity on the eye at the level of the upper orbitals. It is generally smaller than the two other species with this character described here, *M. inthanonensis* sp.nov. and *M. pasiae* sp.nov., with wing length of 2·0–2·4 mm but a positive identification may only be possible from the male genitalia which are illustrated in figures 9 and 10.

The larvae are known to feed in the stems of *Ageratum*, *Bidens* and *Vernonia* but there are probably other hosts in the Compositae.

2.2e *Melanagromyza pasiae sp.nov.* (*figures* 11, 12)

Head: frons broad, 1½ times width of eye, not projecting above eye in profile; orbital bristles strong, 2 equal *ors*, reclinate, 2 *ori* weaker, partially incurved; orbital setulae sparse, reclinate; orbits narrow, distinctly shining; ocellar triangle with apex extending to midway between lower *ors* and upper *ori*; eye large, upright, with conspicuous patch of white hairs at level of *ors*; jowls narrow, 1/8 height of eye, third antennal segment small, round; arista long, in ratio 22:26 with eye height, only finely pubescent.
Mesonotum: 2 strong dorso-centrals, acrostichals numerous, in some 10 rows.
Wing: length in male 2·6 mm; last section of vein M3+4 in ratio 19:29 with penultimate, inner cross-vein slightly beyond midpoint of discal cell.
Legs: mid-tibia with 2 strong lateral bristles.
Colour: frons mat black, orbits weakly, ocellar triangle more strongly shining; mesonotum shining greenish, scutellum more coppery; abdomen shining greenish-coppery; squamae and fringe white, margin only slightly differentiated, very pale brown.
Male genitalia: Aedeagus as in figures 11 and 12, distiphallus strongly pigmented, basal sclerites fused; sperm pump large.
Holotype ♂, N Thailand: Pasia National Park, 500 m, clearing in forest beside road, 30 km NW Mae Hong Son, 28.xi.86; paratype: 1 ♂, same data, to be deposited in British Museum (Nat. Hist.).
Remarks: Although this species generally resembles *M. inthanonensis* sp.nov. the male genitalia are highly distinctive and the two species appear not to be closely related.

2.3 *Melanagromyza pasiensis sp.nov.* (*figures* 13, 14)

Adult: small greenish species, similar in size to *M. metallica* but differing in the bare eye in the male, wing length from 1·75–2·25 mm in male to 2·3 mm in female; indistinguishable on external characters from *M. albisquama* and from *M. erawanensis* sp.nov.
Male genitalia: aedeagus (figures 13 and 14) distinctive.
Holotype ♂, N Thailand: Pasia National Park, 30 km NW Mae Hong Son, clearing in forest at roadside, 500 m, 25.xi.85; paratypes 2 ♂, 2 ♀, same data. Holotype to be deposited in British Museum (Nat. Hist.).
Remarks: Although no apparent differences between this species and *M. albisquama* and *M. erawanensis* sp.nov. are detectable, the male genitalia are entirely distinct.

2.4 *Genus Ophiomyia Braschnikov*

The bean fly, *Ophiomyia phaseoli* (Tryon) (transferred to *Ophiomyia* from *Melanagromyza* by Spencer, 1973), can be a serious pest on beans in the tropics wherever they are cultivated and is well known in Thailand. Greathead (1969) gave a detailed account of its biology. Two further species in this genus can now be recorded for the first time in Thailand.

2.4a *Ophiomyia atralis* (*Spencer* 1961*a*) (*figure* 15)

Bangkok: 16.i.60, empty leaf and stem mine on *Vernonia cinerea* Less.

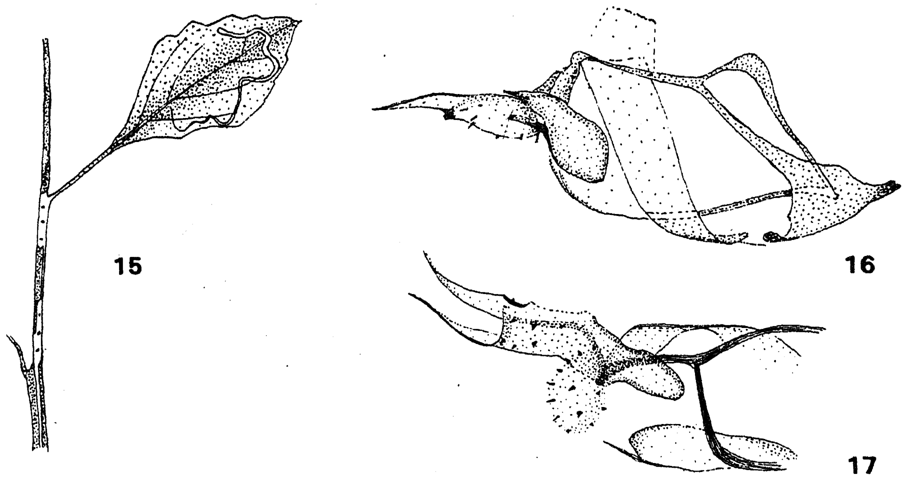
This wide-ranging species was described from Flores, Indonesia and has subsequently been found on many Pacific islands, including New Caledonia, at Darwin, Australia, at Calcutta, India and at Pietermaritzburg, South Africa.

The minute black fly is distinctive in having the squamae and fringe silvery-white (contrast *O. rotata* discussed below). The aedeagus was illustrated by Spencer (1963b: figure 8b). The larva in the first instar forms a narrow leaf mine but then enters a vein and reaches the stem via the midrib where the main mine is formed (figure 15).

2.4b *Ophiomyia conspicua* (*Spencer*)

Thailand: Bangkok, Ja-tu-jak Park, opposite Central Plaza, 1 ♀, 15.xi.85; Chiang Mai, Huay Kaeo Waterfall, 1 ♂, 16.xi.85; University Campus, 1 ♀, 18.xi.85.

O. conspicua is widespread from northern Australia, New Caledonia, Papua New Guinea to India and Sri Lanka. The larvae feed as stem miners on a number of genera of Compositae, particularly *Bidens pilosa* L. and *Eclipta alba* Hassk., also probably *Vernonia cinerea* Less. The adults are small, entirely black, with wing length 1·9–2·3 mm and appear like a typical *Melanagromyza*. However, the male



Figures 15–17. **15.** *Ophiomyia atralis* Spencer; leaf and stem mine on *Vernonia cinerea*. **16–17.** *O. rotata* (Spencer). **16.** Aedeagus, side view. **17.** Ventral view.

genitalia (cf. Spencer 1961a: figure 42) clearly place the species in *Ophiomyia*, to which it was transferred by Spencer (1977a: 349).

2.4c *Ophiomyia rotata* (*Spencer*) (*figures* 16, 17)

Thailand: Bangkok, Ja-tu-jak Park, opposite Central Plaza, 3 ♂, 2 ♀, 15.xi.85.

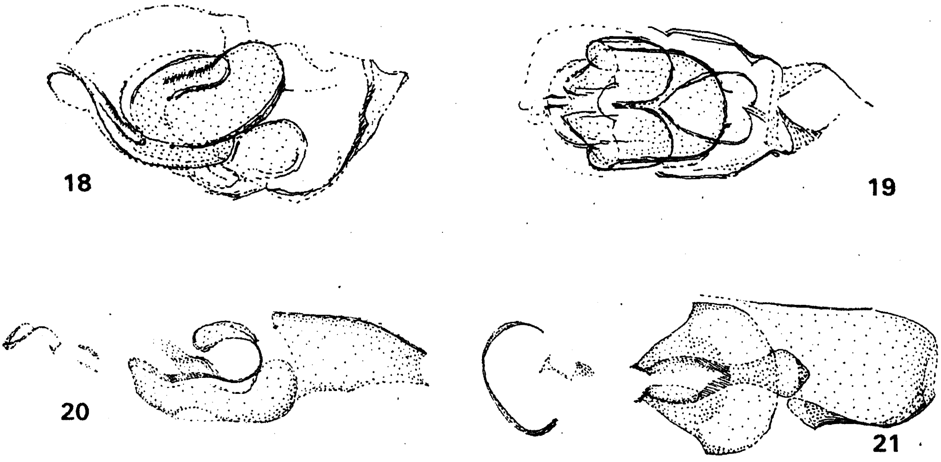
The adult is minute, black, with wing length from 1·5 to 1·8 mm and positive identification will only be possible from the male genitalia which are shown in figures 16 and 17. The genitalia clearly indicate relationship with *O. centrosematis* (de Meij.) which is well known as a minor pest of beans, particularly in East Africa, feeding as an external stem miner (Greathead, 1969; Spencer 1973). It seems virtually certain that *O. rotata* is also a stem-miner on Papilionaceae.

2.5 *Genus Tropicomyia Spencer*

Two species in this genus (Spencer 1973: 180) are now recorded below, one new to science and the other new to Thailand. Leaf mines of 3 further unidentified species are also briefly discussed.

2.5a *Tropicomyia pilosa sp.nov.* (*figures* 18, 19)

Head: frons relatively broad, up to $1\frac{1}{2}$ times width of eye; 2 *ors*, the upper stronger than the lower, both reclinate; 2 *ori*, the upper similar to the lower *ors*, reclinate, the lower weaker, inclined; orbital setulae sparse, 2–4 only, reclinate; jowls exceptionally narrow, 1/15 height of eye; third antennal segment small, round, with a fringe of conspicuous hairs, arista long, equal to height of eye, finely pubescent.
Wing: length 1·6 mm, costa weakening beyond vein R4 + 5, not reaching M1 + 2; last section of M3 + 4 only slightly shorter than penultimate, in ratio 14:18.



Figures 18–21. 18–19. *Tropicomyia pilosa* sp.n. **18.** Aedeagus, side view. **19.** Ventral view. **20–21.** *T. vigneae* (Séguy). **20.** Aedeagus, side view. **21.** Ventral view.

Colour: entirely black, frontal triangle and orbits weakly shining; mesonotum and abdomen brilliantly shining; squamal fringe black.
Male genitalia: aedeagus typical of genus but distinctive, as in figures 20 and 21; sperm pump with very narrow blade.
Holotype: ♂, N Thailand: Mae Chaem, 400 m, SE Doi Inthanon, 100 m from Horticultural Research Station, emerged 29.xi.85 from mine coll. 20.xi. on unidentified bush; paratype: 1 ♀, same data, both to be deposited in British Museum (Nat. Hist.).
Remarks: Distinctive characters of this species are the pilose third antennal segment, long arista and narrow jowls. However, a positive identification, as with most other species in the genus will only be possible from the male genitalia.

A single female from Chiang Mai, Huay Kaeo Waterfall, 17.xi.85, appears to represent a distinct species. The third antennal segment is less pilose, the arista is darker and the jowls broader, 1/8 height of the eye. The status of this specimen can only be clarified when a male can be obtained from the easily accessible locality.

2.5b *Tropicomyia vigneae* (*Séguy*) (*figures* 20, 21)

N Thailand: Erawan Resort, 750 m, 30 km NW Chiang Mai, 1 ♂, 23.xi.85.

This minute black species was described from Madagascar (Séguy 1951) and is widely distributed throughout the Old World tropics from the Cape Verde Islands to Micronesia. The larva is a leaf miner on many genera of Papilionaceae, including *Dolichos*, *Glycine*, *Lablab*, *Phaseolus* and *Vigna*. It was transferred to *Tropicomyia* by Spencer (1973:195) but is not wholly typical of the genus. The leaf mine is epidermal only in the first instar, the larva later forming a normal upper surface linear mine. The larval mouth-hooks do not have the regular serration of typical *Tropicomyia* but have a characteristic arrangement of small teeth (Spencer 1973: figure 295). The puparium does remain in the mine but the posterior spiracles (loc.cit.: figure 296) each have 8–10 pores on a strongly sclerotized projection, while in typical *Tropicomyia* there are 3 larger pores on a flat extension. This is thus clearly an aberrant species but erection of a monotypic genus for it is not desirable at this stage and it is best left in *Tropicomyia* pending further study and the possible discovery of further related species.

2.5c *Unidentified species*

Lasia spinosa Thwaites (Araceae) Bangkok: 16.i.60 A broad, very shallow epidermal mine. This monocot host suggests this is probably an undescribed species.

Canthium horridum Craib (det. Smitinand) (Rubiaceae) Doi Suthep, W Chiang Mai, 1.xii.85. The mines on this small bush are distinctly greenish and more regularly defined than in normal epidermal mines. The puparia at the end of the mines clearly indicate *Tropicomyia* and the species is almost certainly undescribed.

Cryptolepis elegans Wall. (det. Smitinand) (Asclepiadaceae) N Thailand: Mai Chaem, below Doi Inthanon, 21.xi.85. The silvery mines can almost fully cover the large leaves, up to 14 cm long, on this bush. This possibly represents *T. polyphaga* Spencer (1961a) which forms similar mines on *Symplocos spicata* Roxb. (Symplocaceae) and *Tylophora flava* Trimen in Sri Lanka.

2.6 *Genus Amauromyza Hendel*

2.6a *Amauromyza aliena (Malloch)*

N Thailand: Doi Inthanon, 1,350 m, 1 ♂, 20.xi.85.

This species was described from Taiwan (Malloch 1914) and was refound there by Sasakawa (1972) who reared many adults from blotch mines on *Callicarpa formosana* Rolfe (Verbenaceae). It was recorded in Thailand near Fang by Spencer (1962) and the distinctive aedeagus was illustrated (1962: figures 20a, b). The specimens now seen certainly feed on one of the 10 species of *Callicarpa* known in Thailand.

2.7 *Genus Cerodontha Rondani*

Three species, in the subgenera *Cerodontha* and *Icteromyza* are recorded below and two new synonyms are established.

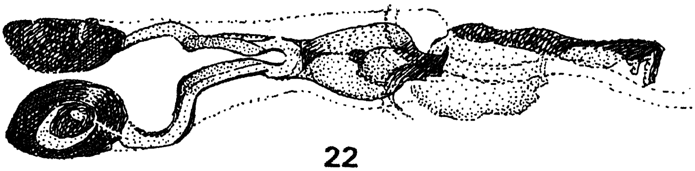
Subgenus *Cerodontha* Rondani

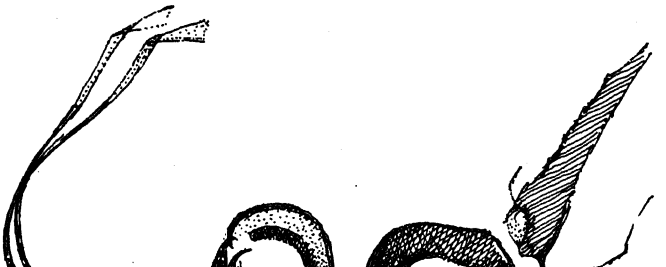
2.7a *Cerodontha (Cer.) ipei Garg*

Cerodontha (Cer.) versicolor Ipe (synonymy established by Sasakawa 1977) (figure 22).

N Thailand: Mae Sa Botanical Gardens, north of Chiang Mai, 660 m, 1 ♂, 27.xi.85.

Spencer (1986) retained *C. versicolor* as distinct from *C. ipei* in view of slight





Figures 22–23. **22.** *Cerodontha*, subgen. *Cerodontha ipei* Garg; aedeagus, ventral view. **23.** *Cerodontha*, subgen. *Icteromyza duplicata* (Spencer); aedeagus, side view.

apparent differences in the male genitalia as illustrated by Ipe (1971: figure 3e) and Garg (1971: figure 2e), and the wide separation of the only two known populations in the Himalaya (*ipei*) and the high ranges of Kerala (*versicolor*). In the further specimen now seen from northern Thailand the genitalia agree exactly with those of *versicolor* from Kerala and now I have no hesitation in accepting Sasakawa's synonymy. The distiphallus is not solid, as appears from both Garg's and Ipe's illustrations but is essentially hollow, with a small central area of dark pigmentation surrounded by a lighter area which is again surrounded by a darker area (figure 22).

This species represents an interesting case of wide distribution both from west to east and north to south and is doubtless present at intermediate, high altitude areas between the high mountains of northern and southern India.

Subgenus *Icteromyza* Hendel

Two species are recorded below from Bangkok and N Thailand. There has been confusion in the past over the status of *Cerodontha* (*Ict.*) *duplicata* Spencer (1961a) and, with the additional material now seen, it is hoped that the species has at last been finally clarified. Two new synonyms are established and one species in India is resurrected.

2.7b *Cerodontha* (*Ict.*) *piliseta* (*Becker* 1903)

Cerodontha (*Ict.*) *hirsuta* Sasakawa (1972), syn.nov.

Bangkok: Ja-tu-jak Park, damp ground, 1 ♀, 15.xi.85

Described from Egypt, this species is now known at many localities from the Afrotropical and Oriental Regions to N. Australia and Micronesia, and has also been recorded in Taiwan (Sasakawa 1977). It is distinguishable by the conspicuously yellow femora, pilose eyes and mat greyish-black mesonotum.

Sasakawa (1972:62) described *hirsuta* from a single male from Taiwan, citing as differentiating characters from *piliseta* the "smaller size", "densely gray-dusted mesonotum" and the "hairy eye". Wing length of *hirsuta* is given as 1·4 mm; I have before me a male of *piliseta* from Yap Is., Federated States of Micronesia, which is even smaller, with wing length of 1·25 mm. The mesonotum in typical *piliseta* is greyish-black; it could equally well be described as "gray-dusted". In all *piliseta* I have seen the eye is "hairy", with some variation in the degree of pilosity. I therefore formally synonymise *C.* (*I.*) *hirsuta* Sasakawa with *C.* (*I.*) *piliseta* (Becker) herewith. It is unfortunate that the genitalia of *hirsuta* were not examined at the time of its description, when its true identity would have become apparent.

2.7c *Cerodontha* (*Ict.*) *duplicata* (*Spencer* 1961*a*) (*figure* 23)

Cerodontha (*Ict.*) *floresensis* (Spencer 1961a) (synonymy established by Sasakawa, 1972)
Cerodontha (*Ict.*) *hardyi* (Sasakawa 1963b), syn. nov.

N Thailand: Chiang Mai, 1 ♂, 17.xi.85; Doi Inthanon, 1 ♂, 20.xi.85; Mae Sa Botanical Gardens, 1 ♂, 1 ♀, 22–24.xi.85; Bang Tong Forestry Station, 1,000 m, NW Mae Hong Son, 1 ♀, 27.xi.85; Doi Suthep, 1 ♀, 2.xii.85.

There has been confusion about the status of this species. *C. duplicata* was described from Flores, Indonesia (Spencer 1961a) and was recorded from Nepal by Spencer (1965a). It was also recorded from Taiwan by Sasakawa (1972) who synonymised with it *C. floresensis* Spencer (1961a) also described from Flores. Sasakawa (1963b) described *C. hardyi* from Irian Jaya and this species was later recorded by Spencer (1977a) from Papua New Guinea. *C. floresensis* was later recorded from the Philippines (Spencer 1965b) and from New Britain (Spencer 1966a).

With the considerable amount of material now available, it is clear that there is minor variation both in the frons colour and also in the size. Spencer (1961a) illustrated the genitalia of the holotypes of both *duplicata* and *floresensis* and there appeared to be slight differences in the curvature of the aedeagus but the actual distal processes are virtually identical. The aedeagus of the male from the Mai Sa Botanical Gardens is shown in figure 23. It will be seen that this agrees almost exactly with the earlier drawings of *C. duplicata* (Spencer 1961a) from Flores, with *C. hardyi* (Sasakawa 1963b) from Irian Jaya and with *C. hardyi* (Spencer 1977a) from Papua New Guinea.

The synonymy of *C. floresensis* with *C. duplicata* established by Sasakawa (1972) is now accepted. Sasakawa (1977) also synonymised 3 species described from northern India with *duplicata*—*C. keethamensis* Garg (1971); *C. rishii* Garg (1971); and *C. periaryi* Singh and Ipe (1973). The status of *C. keethamensis* and *C. periaryi* cannot be confirmed, as both species are only known from females. However, the genitalia of *C. rishii*, illustrated by Garg (1971: figure 7e), show this to be clearly distinct from *C. duplicata* and this species is resurrected. The genitalia of a male from Nepal, recorded as *C. duplicata* by Spencer (1965a) have now been examined and the aedeagus agrees exactly with that of *rishii*. It is thus clear that in this complex of species there is one in northern India and Nepal, *C. rishii*, with a second wide-ranging species, *C. duplicata*, extending from Thailand and Taiwan to Indonesia and Papua New Guinea, a third, *C. nigricoxa* (Malloch 1914) known only from Taiwan (Spencer 1962b: figure 21) and a fourth, *C.* (*I.*) *triplicata* Spencer (1977c) in Australia (New South Wales and Western Australia). The host of *C. triplicata* was confirmed as *Juncus pallidus* R. Br. and it can be assumed that the other 3 species also all feed on *Juncus* spp.

2.8 *Genus Liriomyza Mik*

Three species are discussed below, all representing new records for Thailand. *L. chinensis* (Kato) is an important pest of onions.

2.8a *Liriomyza brassicae (Riley)*

? Bangkok area: 1985, intercepted in London, larvae mining Chinese cabbage ('pakchoi'), 1 ♂, 1 ♀ reared (P Seymour, MAFF).

This is a semi-cosmopolitan species which is widespread in India and known in Malaysia but not previously recorded in Thailand.

The larvae feed primarily on many genera of Cruciferae. A further favourite host is *Tropaeolum* (nasturtium) and the fly is invariably present wherever this ornamental is cultivated. Adults are not readily distinguishable from *L. compositella* Spencer (see below) but the male genitalia are distinctive (cf. Spencer 1973: figures 219a, b).

2.8b *Liriomyza chinensis* (*Kato*)

Dizygomyza cepae ssp. ***chinensis*** Kato (1949: 12) (figures 24, 25)

This onion leaf miner was originally described from Inner Mongolia and Peking and has since been recorded in Japan, Malaysia and Singapore (Spencer 1973: 164). Surprisingly it has also recently been discovered attacking onions in the south of France (Martinez 1982). It is now well known in vegetable growing areas near Bangkok and can cause considerable damage to young onions.

The species is readily recognisable by the angulate third antennal segment and belongs to the small group in *Liriomyza* in which the scutellum is gray, not yellow as is normal and this is shown in figures 24 and 25.

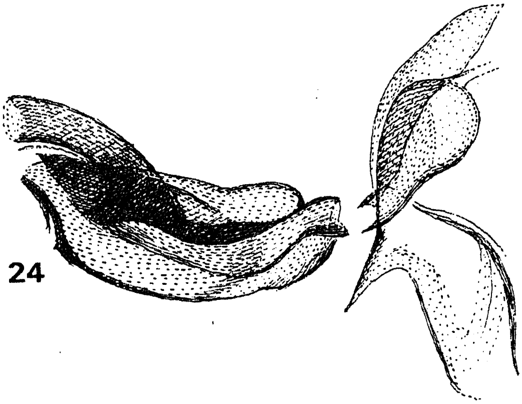
2.8c *Liriomyza compositella Spencer* (1961*a*)

N Thailand: Doi Suthep, W Chiang Mai, 1 ♀, 4.xii.85; Erawan Resort, N Chiang Mai, 1 ♂, 1 ♀, 23.xi.85; Pasia N P, NW Mae Hong Son, 1 ♂, 29.xi.85. Empty leaf mines also found on *Crassocephalum crepidioides* S Moore, Chiang Mai, Arboretum, 26.xi.85 and at Forestry Station, near Hong Tong, NW Mae Hong Son, 28.xi.85; on *Synedrella nodiflora* Gaertner, Chiang Mai, Arboretum, 25.xi.85; Doi Suthep, 4.xii.85; and Pasia N P, 28.xi.85; and on *Tithonia diversifolia* Gray, Doi Inthanon, 20.xi.85. Also a previously unpublished record from Malaysia, Ulu Gombak, 25 km N Kuala Lumpur, 3.xii.75, on *Crassocephalum crepidioides* (KAS).

Widespread in Oriental Region, recorded from Taiwan, Hong Kong, India (Delhi, Bombay, Bangalore), Sri Lanka. Additional hosts are *Callistephus chinensis* Nees, *Solidago canadensis* L. and probably *Vernonia cinerea* Less. (all India). The larva forms an irregular upper surface linear mine, which sometimes may begin with a spiral.

2.9 *Genus Phytoliriomyza Hendel*

Two species, both originally described from Australia and both new to Thailand, are recorded below.



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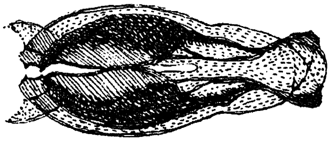

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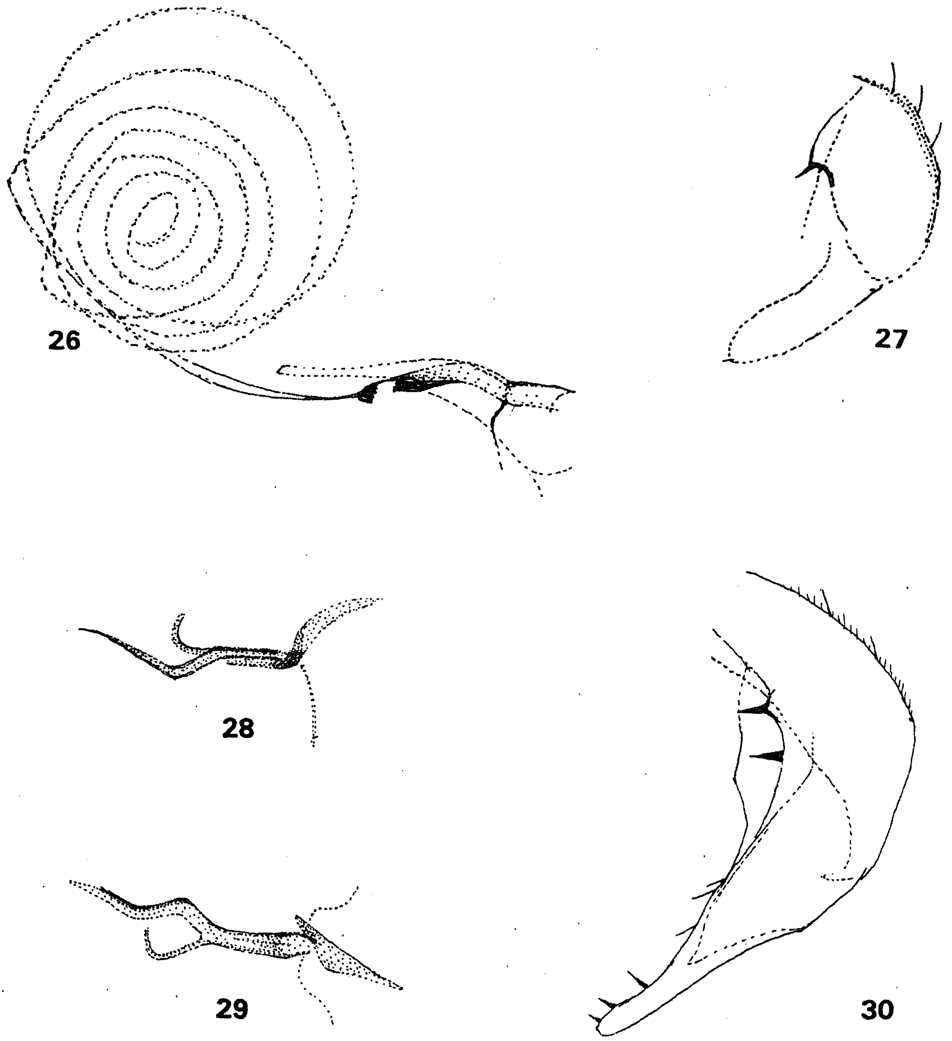
Figures 24–25. *Liriomyza chinensis* (Kato). **24.** Aedeagus, side view. **25.** Distiphallus, ventral view.

2.9a *Phytoliriomyza australensis Spencer* (1963*a*) (*figures* 26, 27)

N Thailand: Doi Inthanon, road to Mae Chaem, 1,000 m, 1 ♀, 20.xi.85; roadside, 2,100 m, 1 ♀, 3.xii.85; summit, 2,590 m, 1 ♀, 3.xii.85.

Known in Australia from NSW and ACT, subsequently recorded in Nepal (Spencer 1965a: 30) and Sri Lanka (Spencer 1975: 216), the discovery of this species in Thailand partially fills the gap in its known distribution. It can be confidently predicted that it is present also in mountainous areas in Sabah, Indonesia and probably Papua New Guinea.

In the male genitalia the aedeagus ends in a long, fine tubule (figure 26) and the surstyli are oval, discrete, indicating close relationship with the widespread *P. arctica*



Figures 26–30. **26–27.** *Phytoliriomyza australensis* Spencer. **26.** Aedeagus, side view. **27.** Surstylus. **28–30.** *P. tricolor* (Mall.). **28.** Aedeagus, side view. **29.** Ventral view; **30.** Surstylus.

(Lundbeck) (nearest known locality: southern India). The third antennal segment is small, round, with a fringe of conspicuous white pubescence; wing length is 1·6 mm, with the discal cell small and the last section of vein M3+4 twice the length of the penultimate section; the palps are slender and pale, yellow or yellowish-brown.

In a further female from Doi Inthanon, 2,100 m, 3.xii.85, the palps are enlarged and deep black. Although closely resembling typical *australensis*, it is believed that this must represent a distinct species but its status can only be clarified when a male becomes available.

2.9b *Phytoliriomyza tricolor* (*Malloch* 1927) (*figures* 28–30)

N Thailand: Bang Tong Forestry Station, 1,000 m, 50 km NW Mae Hong Son, 1 ♂, 27.xi.85; Doi Suthep, W Chiang Mai, 1,000 m, 4.xii.85.

This species has hitherto only been known in Australia, where it is widespread in southern States from New South Wales to Western Australia (Spencer 1977c). Its discovery in Thailand represents an unexpected extension of its range but this is paralleled by *P. australensis* recorded above.

P. tricolor belongs to the small group with asymmetrical distal tubules in the aedeagus (figures 28, 29) and the surstyli are distinctive, being fully fused with the epandrium and long and narrow (figure 30), sperm pump minute. The third antennal segment is longer than broad, virtually bare; wing length is 1·6 mm.

A closely-related species, *P. nepalensis* Spencer (1977b) is only known from Nepal. The aedeagus is very similar to that of *P. tricolor* (Spencer 1977b: figures 1, 2) but the epandrium is distinctively different, bearing a group of some 15–20 strong spines behind the surstyli which are divided from the epandrium by a suture and are large, oval, with 1 spine and several hairs along their inner margin.

2.10 *Genus Pseudonapomyza Hendel*

Four species are now known in Thailand, of which one is described below as new to science and the synonymy of *P. santokhi* (Garg) from India is established with *P. atrata* (Malloch).

P. spicata (Malloch 1914) has been recorded in the Bangkok areas as a leaf miner on maize (*Zea mays* L.). Unfortunately it has not been possible to examine any specimens to confirm this identification. Recent work has shown that *P. spinosa* Spencer (1973) is more common than *P. spicata* in much of the Oriental Region, including India and also in Northern Australia and the Pacific. The two species were discussed by Spencer (1973) and their very different genitalia are shown in figures 31, 32 (*spicata*) and figures 33, 34 (*spinosa*).

2.10a *Pseudonapomyza alternantherae* (*Séguy* 1951) (*figures* 35–38)

Bangkok: empty leaf mines on *Alternanthera sessilis* DC., 16.i.60; Chiang Mai, Huay Kaeo Arboretum, 1 ♂, 6.xii.85.

This species was described from Madagascar and has subsequently been found in Cameroun, South Africa (Cape Province) and India (Bombay, Calcutta). The type

2.9a *Phytoliriomyza australensis Spencer* (1963*a*) (*figures* 26, 27)

N Thailand: Doi Inthanon, road to Mae Chaem, 1,000 m, 1 ♀, 20.xi.85; roadside, 2,100 m, 1 ♀, 3.xii.85; summit, 2,590 m, 1 ♀, 3.xii.85.

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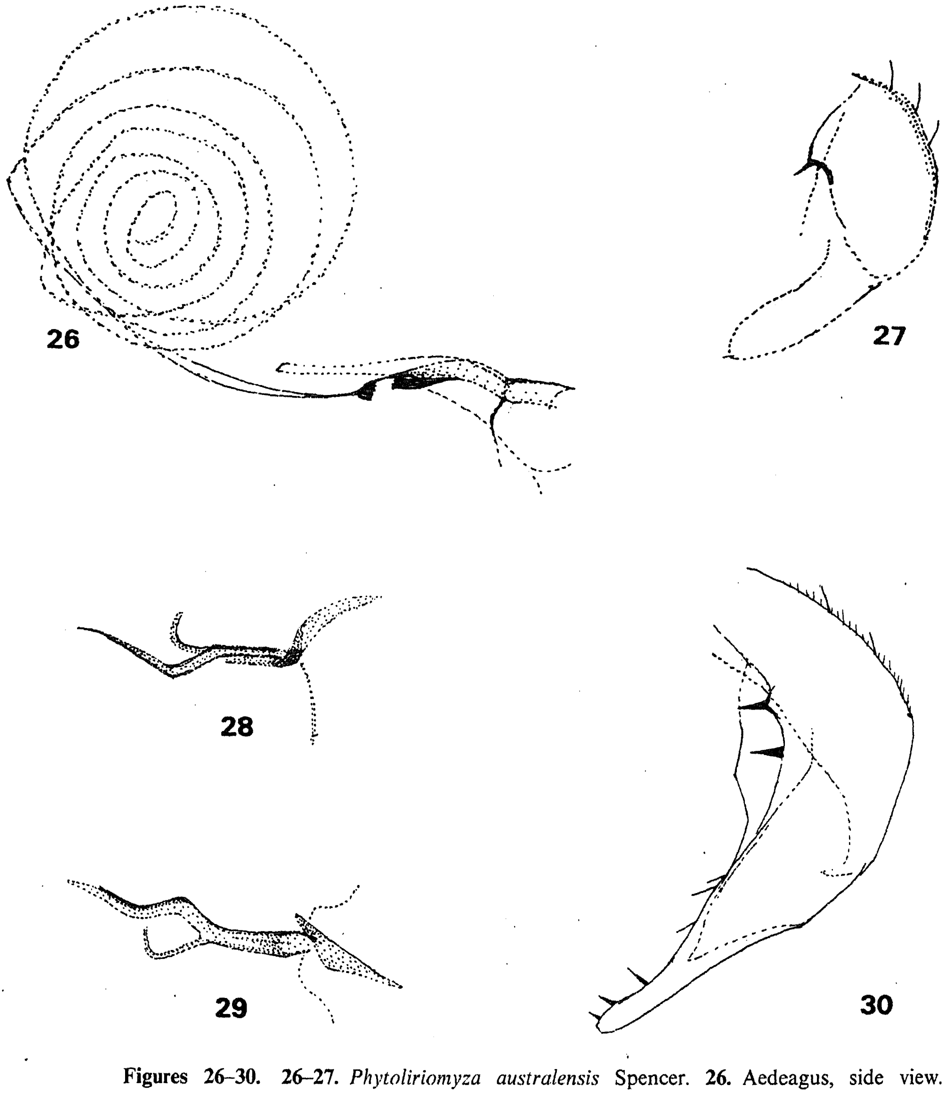

Figures 26–30. 26–27. *Phytoliriomyza australensis* Spencer. **26.** Aedeagus, side view. **27.** Surstylus. **28–30.** *P. tricolor* (Mall.). **28.** Aedeagus, side view. **29.** Ventral view; **30.** Surstylus.

(Lundbeck) (nearest known locality: southern India). The third antennal segment is small, round, with a fringe of conspicuous white pubescence; wing length is 1·6 mm, with the discal cell small and the last section of vein M3+4 twice the length of the penultimate section; the palps are slender and pale, yellow or yellowish-brown.

In a further female from Doi Inthanon, 2,100 m, 3.xii.85, the palps are enlarged and deep black. Although closely resembling typical *australensis*, it is believed that this must represent a distinct species but its status can only be clarified when a male becomes available.

2.9b *Phytoliriomyza tricolor* (*Malloch* 1927) (*figures* 28–30)

N Thailand: Bang Tong Forestry Station, 1,000 m, 50 km NW Mae Hong Son, 1 ♂, 27.xi.85; Doi Suthep, W Chiang Mai, 1,000 m, 4.xii.85.

This species has hitherto only been known in Australia, where it is widespread in southern States from New South Wales to Western Australia (Spencer 1977c). Its discovery in Thailand represents an unexpected extension of its range but this is paralleled by *P. australensis* recorded above.

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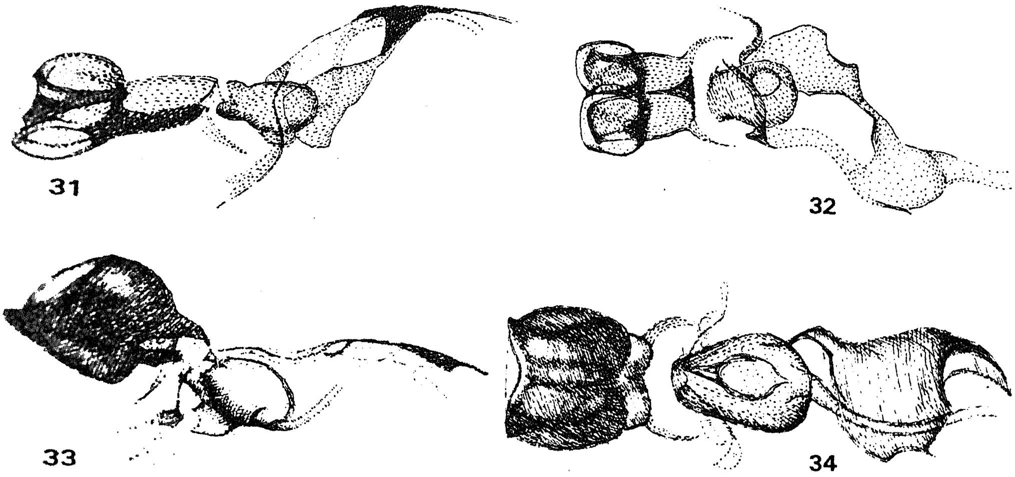
2.10a *Pseudonapomyza alternantherae* (*Séguy* 1951) (*figures* 35–38)

Bangkok: empty leaf mines on *Alternanthera sessilis* DC., 16.i.60; Chiang Mai, Huay Kaeo Arboretum, 1 ♂, 6.xii.85.

This species was described from Madagascar and has subsequently been found in Cameroun, South Africa (Cape Province) and India (Bombay, Calcutta). The type



Figures 31–34. 31–32. *Pseudonapomyza spicata* (Mall). 31. Aedeagus, side view. 32. Ventral view. 33–34. *Pseudonapomyza spinosa* Spencer. 33. Aedeagus, side view. 34. Ventral view.

and the series from Cameroun were reared from *A. sessilis* and the specimens obtained in India were reared or caught on the related host *Achyranthes aspera* L. (both Amaranthaceae).

P. alternantherae belongs to the group with the third antennal segment round and the squamal fringe black, in contrast to the grass-feeders, in which the third antennal segment is angulate and the squamal fringe normally silvery-white (cf. *P. rampae* described below). In this species, as in most, the outer cross-vein is lacking. The wing was illustrated by Séguy (1951: figure 5).

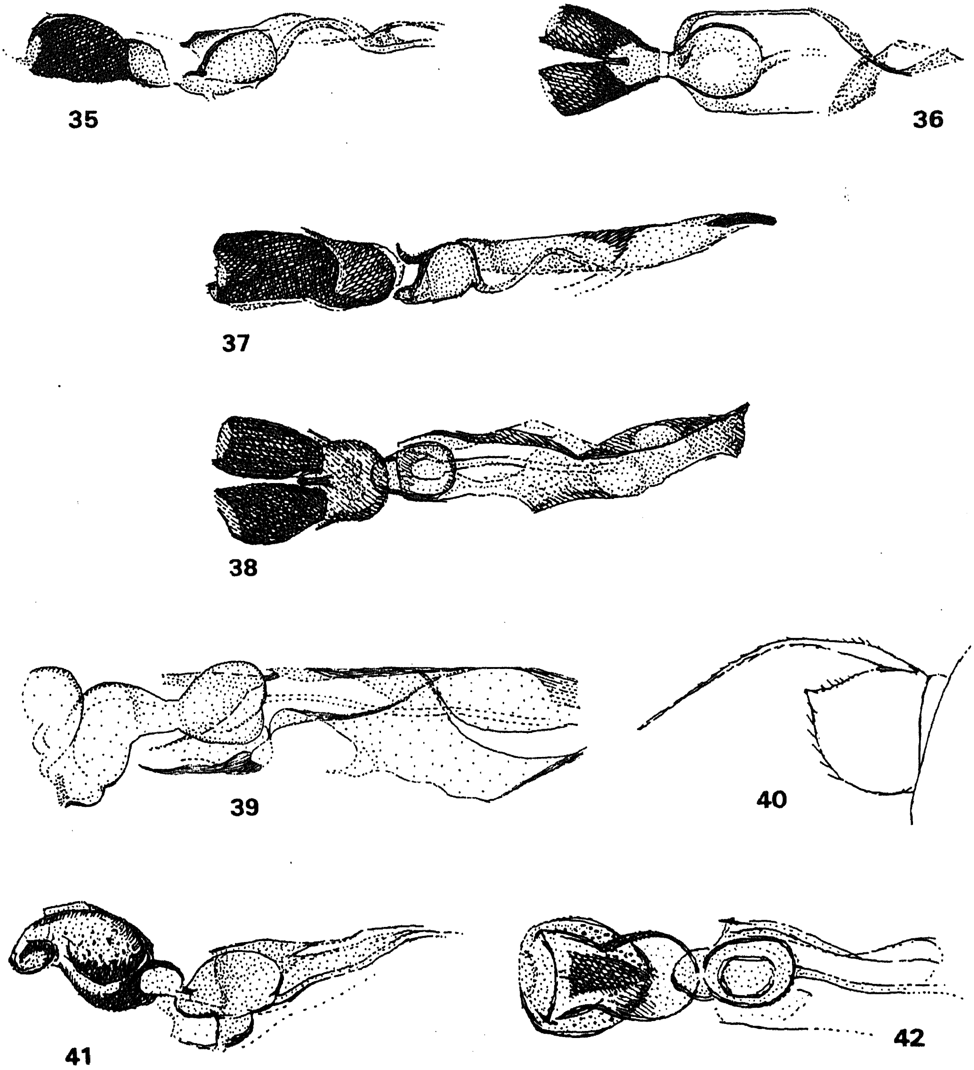
The male genitalia have been examined of the holotype from Madagascar and of specimens from Bombay, Calcutta, Cameroun and South Africa, in addition to the one from Chiang Mai. The general form of the aedeagus is similar in all populations and closer than in any pair of species known in the genus, with no external differences in the adults.

Three forms are detectable:

(i) Madagascar and Mt. Cameroun, Cameroun, West Africa. Here, the process immediately behind the paired tubules of the distiphallus is small (figure 35), the bladder further to the rear is large and almost circular and the basal sclerites are slender and relatively short (figure 36). Specimens reared from *Alternanthera sessilis* (Amaranthaceae).

(ii) South Africa (Cape Province). A single caught specimen and the genitalia were illustrated by Spencer (1961b: figures 18a, b). The distal tubules are more slender and the central bladder is small. This is close to form 1.

(iii) India (Bombay, Calcutta), N Thailand. Indian specimens were caught on and reared from *Achyranthes aspera* L. (Amaranthaceae). The single specimen from Thailand was caught, with no indication of the host. The genitalia (figures 37 and 38) are identical in the 3 populations from Bombay, Calcutta and N Thailand. The process behind the distal tubules is large and the central bladder small and more



Figures 35–42. 35–38. *Pseudonapomyza alternantherae* (Séguy). **35.** Aedeagus, side view. **36.** Ventral view (holotype, Madagascar) **37.** Aedeagus, side view. **38.** Ventral view (Thailand). **39.** *Ps atrata* (Mall.); aedeagus, side view. **40–42.** *Ps. rampae* sp.n. **40.** Third antennal segment. **41.** Aedeagus, side view; **42.** Ventral view.

elongate; the basal sclerites are longer and broader. The whole aedeagus is generally larger than in form 1.

The population at Bangkok feeding on *Alternanthera sessilis* is obviously of great importance for the further clarification of this complex. If the genitalia are as in the type from Madagascar, then it would appear that form 3 feeding on *Achyranthes aspera* could merit separate specific status, and forms 1 and 3, each with their own host are both present in Thailand. However, if the genitalia are as in form 3, then the two different hosts, *Alternanthera* and *Achyranthes*, closely related genera in the

Amaranthaceae, do not provide any evidence supporting separate specific status of forms 1 and 3.

It will clearly be of great interest to study additional reared material of this complex from the two known and possibly other hosts and from further areas in the oriental and afrotropical regions but for the time being I propose to treat these populations as representing a single species but with divergence in the genitalia indicating incipient speciation.

2.10b *Pseudonapomyza atrata* (*Malloch* 1914) (*figure* 39)

Phytagromyza tibialis Sasakawa (1963a) (synonymy established by Spencer 1965c)
Phytagromyza santhokhi Garg (1971), syn. nov.
N Thailand: Doi Suthep, 1 ♀, 14.xi.57 (J L Gressitt).

This wide-ranging oriental species, described from Taiwan and subsequently recorded in Vietnam, Indonesia and India, belongs to the same group as *P. alternantherae*, with the third antennal segment round and the squamal fringe dark, brown or blackish. It differs from *P. alternantherae* in having a complete discal cell, with the outer cross-vein in continuation of the inner, and the aedeagus (figure 39) is distinctively different from that of *P. alternantherae*.

The specimen from Thailand was caught on bamboo and this led Sasakawa (1963a: 47) to suggest that the species is a leaf miner on bamboo. This cannot be excluded but it does not belong to the group feeding on Gramineae, which have the third antennal segment angulate and the squamal fringe silvery-white. The male genitalia indicate association with the group feeding on Acanthaceae and Amaranthaceae.

Sasakawa (1977: 268) in the Catalog of oriental Agromyzidae retained *tibialis* as a distinct species, even treating it as a new combination in *Pseudonapomyza*, ignoring my earlier discussion and synonymy (Spencer 1966a: 516). The differences from *P. atrata* cited by Sasakawa when describing *tibialis* were the smaller size (wing length 1·9 mm), 3 pairs of strong dorso-central bristles and 3 strong mid-tibial bristles. When establishing the synonymy of *tibialis* with *atrata* I examined the 5 specimens in the type series of *atrata* and found the wing length to range from 1·75–2·3 mm, all specimens have 3 strong dorso-centrals and there is variation in the number of mid-tibial bristles from 1–5, even differing on the two legs of the same specimen. The average on 10 legs is exactly 3. Thus all differentiating characters cited by Sasakawa in fact apply to *atrata*. I have now also examined the holotype of *P. tibialis* and have no hesitation in confirming the synonymy already established.

Garg (1971) described *Phytagromyza santokhi* from Uttar Pradesh, northern India and this was synonymised with *P. alternantherae* by Sasakawa (1977: 267). However, Garg's illustration of the male genitalia (1971: figure 3i) and also the presence of a complete discal cell with vein m-m present shows this to be synonymous with *P. atrata* and the synonymy of *P. santokhi* with *P. alternantherae* established by Sasakawa (1977: 267) is rejected as incorrect.

2.10c *Pseudonapomyza rampae sp.nov.* (*figures* 40–42)

Head: frons narrow, equal to width of eye; 2 *ors* and 2 *ori*, the upper *ors* reclinate, the lower 3 inclined; orbital setulae minute, sparse, reclinate; jowls extended at rear, about 1/3 vertical height of eye; third antennal segment finely angulate (figure 40).

Mesonotum: 3 dorso-centrals, the 3rd small, half length of second; acrostichals in 4/5 irregular rows.

Wing: length in male 1·4 mm, in female 1·4–1·5 mm; costal sections 2,3,4 in ratio 17:9:13 (holotype), 20:10:12 in one female paratype, second section thus relatively ong, almost twice length of third.

Colour: frons brownish-black, jowls dark brown, all antennal segments black; mesonotum deep black, moderately shining, abdomen strongly shining black; legs entirely black; squamae and fringe silvery-white.

Male genitalia: aedeagus distinctive, as in figures 41, 42; sperm pump large, equal in ength to aedeagus.

Holotype ♂, N Thailand: Chiang Mai, Huay Kaeo Experimental Cattle Farm, 5.xii.85, swept on grass; paratypes: 2♀, same locality, 21.xi.85; 1♀, Chiang Mai, University Campus, 18.xi.85. Holotype and paratypes in British Museum (Nat. Hist.).

In one female, Chiang Mai, Arboretum, 17.xi.85, the third antennal segment is less finely angulate and the squamal fringe is dark, ochrous-brown and this probably represents another species but its status cannot be further clarified at this time.

Remarks: On external characters I can find no difference between this species and *P. spicata*. The genitalia of the two species, however, are entirely distinct (cf. figures 31 and 32).

I have pleasure in dedicating this new species to Rampa Rathanarithikul, without whose help my visit to Thailand might well not have taken place.

3. Conclusion

The material studied indicates the wide range of some species in the oriental region from southern India to northern Thailand, and the even wider range of several species, extending from Thailand to Java, the Philippines and Australia. This confirms the great mobility previously noted in the Agromyzidae, particularly their ability by long-range aerial dispersal to cross substantial water gaps of up to 800 km, for example from Caribbean islands to southern Florida (Spencer and Stegmaier 1973) and from eastern Australia to New Zealand (Spencer 1976).

While in most species examined the male genitalia are virtually identical even in widely separated populations as far apart as southern India and Thailand, and Thailand, Java and the Philippines, in the case of *Pseudonapomyza alternantherae* there is clear evidence of incipient speciation in the 5 populations examined from South Africa to Thailand, this being most developed between the populations in Africa and Madagascar on the one hand and in India and Thailand on the other.

Acknowledgements

My collecting in Thailand was only possible with the help of too many people to name individually but I must particularly thank the following:

Manop and Rampa Rathanarithikul for assistance both in Bangkok and Chiang Mai; Jittawadee Rodcharoen for help with transport and collecting in Bangkok; Chamlong Phenklai, Director of the Royal Forest Department Herbarium at

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I must thank Dr T van Leeuwen, Zoologisch Museum, Amsterdam for the loan of types of *Agromyza panici* de Meij. and Dr L Matile, Muséum d'Histoire Naturelle, Paris for the loan of the holotype of *Pseudonapomyza alternantherae* (Séguy).

I would like to express my appreciation and thanks to the Trustees of the British Museum (Natural History) for facilities for studying types and other material. The willing help of the staff of the Diptera Section during my frequent visits to the BM is greatly appreciated.

Finally, I must thank my wife Ann who prepared all the illustrations so essential for the description of new species in the Agromyzidae.

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Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions

K VENKATARAMAN and S KRISHNASWAMY*

School of Energy, Environment and Natural Resources and *Department of Environmental Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. Experiments were conducted with *Anisops bouvieri* as predator to study the advantages of helmet development in cyclomorphic form *Daphnia cephalata* King and the results were compared with another non-helmeted *Daphnia similis* Claus. The helmeted *Daphnia cephalata* avoided *Anisops bouvieri* predation better than the non-helmeted form *Daphnia similis*. The predator selectively preys upon smaller sized *Daphnia similis* than the larger *Daphnia cephalata*.

Keywords. *Anisops bouvieri* predation; advantages of cephalic expansion; *Daphnia cephalata* King; *Daphnia similis* Claus.

1. Introduction

Cyclomorphosis is a phenomenon which involves a dorso-anterior expansion of the cephalic area so that a laminate crest is produced in some species of the cladoceran genus *Daphnia*. It is believed so far that due to the influence of environmental factors all members of a population possessing the same body form vary it over the year. The adaptive significance of such phenotypic changes has been argued for many years (Coker and Addlestone 1938; Brooks 1946, 1947; Hrbacek 1959; Jacobs 1961; Hebert 1978a, b). Until recently, the cephalic expansion (helmet) in *Daphnia* was related to all possible field as well as the laboratory factors, since the degree of change in size of the cephalic region, the body and the tail spine is variable in different populations of the same species. At present, suggestions are that crest development is a predation avoidance mechanism, evation being the most important factor (Grant and Baily 1981). The original theory on the cephalic expansion as a function to lessen sinking rates in less dense or viscous water has little credence. The present study on this aspect focuses on the advantage of helmet development on *Daphnia cephalata* King and the impact of predation on *D. similis* Claus by a hemipteran (Notonectidae) predator *Anisops bouvieri*, to substantiate the theory proposed by Dodson (1974) that the mortality due to vertebrate and invertebrate predators may be reduced by a change in morphology of the prey species.

2. Material and methods

To study the advantage of helmet development on *D. cephalata* and the impact of predation on *D. similis*, parthenogenetic females were separated from the field collections. *A. bouvieri* (predator) which had been netted from the same ponds where the daphnids were collected, were conditioned to the laboratory by feeding them with both the species for a few days and then starving the predator for 36 h before an experiment. All the experiments were carried out under the laboratory illumination in tap water and it was quite easy to observe the activities of the predator because they hang motionless in the water until a prey comes within close range, eliciting an attack.

To study the effect of prey density on predation, *A. bouvieri* (adult and young) were offered *D. similis* in three 500 ml beakers in 50, 100 and 200 concentrations. Another series of test individuals were provided with *D. cephalata* in the same densities as mentioned above. Experiments were repeated thrice and 90 min were given for each experiment.

To study the prey preference, a predator was offered equal number of (10 + 10) both *D. similis* and *D. cephalata* for a period of 90 min. Throughout the experiment, the prey density was kept constant by introducing a prey as and when it was preyed.

3. Results and discussion

The first set of experiments dealing with the effect of density on predation, shows that adult *A. bouvieri* preyed more *D. similis* (figure 1, a = 0·439) than the young (a = 0·162) and with increase in the density of the prey, predation also increased (adult $P < 0{\cdot}001$, highly significant; young $P < 0{\cdot}001$, highly significant). In experiments using *D. cephalata* also, the number of prey consumed by the predator increases with the increasing density of prey (young $P < 0{\cdot}001$, highly significant; adult $P < 0{\cdot}05$ significant) (figure 2). Comparison of both the prey species reveals that *D. similis* is more susceptible than the helmeted *D. cephalata* for *A. bouvieri* predation (figure 3).

The study on prey preference shows that the number of *D. similis* consumed by *A. bouvieri* is more than that of *D. cephalata* ($P < 0{\cdot}001$, highly significant, figure 4). An average of 45 *D. similis* were preyed by *A. bouvieri* which is approximately 3 times

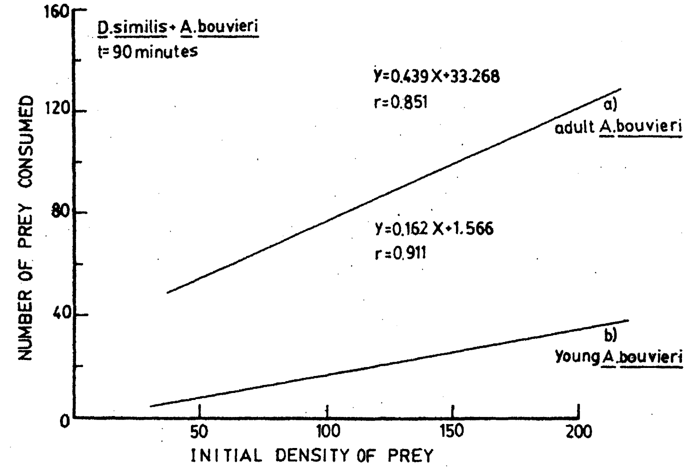

Figure 1. The relationship between the density of *D. similis* and the number of prey consumed by *A. bouvieri* (adult and young).

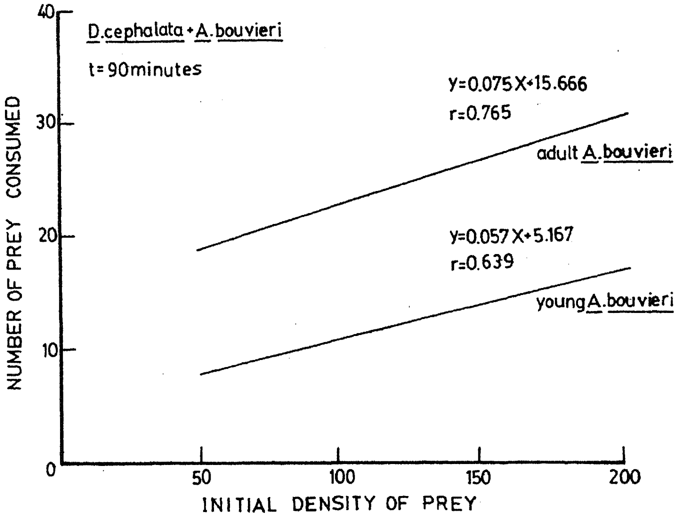

Figure 2. The relationship between the density of *D. cephalata* and the number of prey consumed by *A. bouvieri* (adult and young).

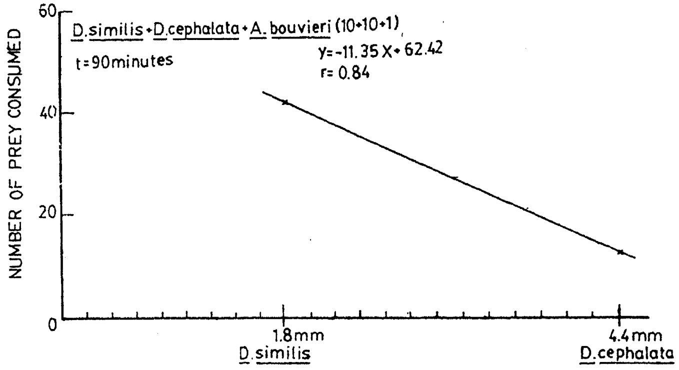

Figure 3. Histogram showing the number of prey consumed by adult and young *A. bouvieri* at 3 different densities of *D. similis* and *D. cephalata*.

higher than the predation of *D. cephalata* (16) (table 1). It is evident that the helmeted *D. cephalata* is less susceptible to *A. bouvieri* predation than *D. similis*.

O'Brien and Vinyard (1978) observed that during predation experiments with *A. bouvieri*, the crested *D. cephalata* better avoided predation than did the uncrested *D. similis*. O'Brien *et al* (1980) stated that the crest on *D. longiremis* f. *cephalata* protected it from invertebrate predation without altering the visual body size. Jacobs (1966) has also shown that the helmeted morph of *D. galeata mendotae* was preyed upon less than the non-helmeted morph. O'Brien *et al* (1980) also supported the theory that the presence of helmet is advantageous for the prey, *D. longeremis* f. *cephalata* and further stated that the presence of crest is an example of ingenuity of natural selection in equipping animals to survive in the face of selective pressures.

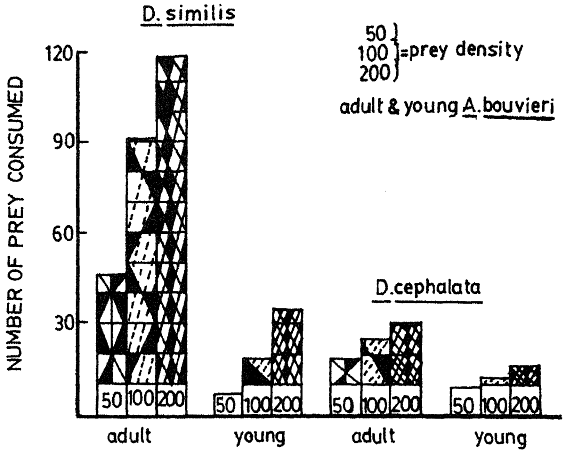

Figure 4. The relationship between the number of prey consumed by *A. bouvieri* (adult) exposed to equal number of both *D. similis* and *D. cephalata* (10 + 10).

Table 1. Predation experiments in which *A. bouvieri* was exposed to both *D. similis* and *D. cephalata* (density = 10 each) for 90 min. The density of the prey species was maintained constant by adding as and when one prey was killed.

Experiment No.	Number of *D. similis* preyed	Number of *D. cephalata* preyed	Number of observations
1	32·33 ± 3·51	8·33 ± 2·52	3
2	57·66 ± 5·86	23·30 ± 5·50	3
Average	45·00	16·00	6

Likewise, the presence of 'Nackenzahne' is the only protective mechanism in *D. minnehaha* to reduce the predator's (*Chaoborus*) handling efficiency (Krueger and Dodson 1981).

The same mechanism may be involved in the reduction of predation on exuberent varieties of other zooplankton species. In Gilbert's (1963) study of the rotifer *Branchionus* sp. short spined parental forms suffered greater predation than their offspring with long predator-induced spines. Zaret (1972a, b) reported that the exuberent horned morphs of *Ceriodaphnia cornuta* was preyed upon less often by the fish predator. Kerfoot (1977) and O'Brien *et al* (1979) showed that some of the exuberent structures of *Bosmina* sp. reduce predation by copepods.

Thus, the presence of helmet in *D. cephalata* may be advantageous to avoid predation than the non-helmeted *D. similis* and acts as an 'antilock and key' as suggested by Dodson (1974), O'Brien and Vinyard (1978) and O'Brien *et al* (1980).

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Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson)

S AJMALKHAN, K RAJENDRAN and R NATARAJAN
Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, India

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Abstract. The 96 h LC_{50} value for zinc was 100 ppb. Larvae reared in 7 sublethal (1, 5, 10, 30, 50, 70, 90 ppb) and 96 h LC_{50} level concentrations (100 ppb) showed survival rates more than 10%, thus perfectly fitting in with definition of chronic concentration. Survival rate of larvae decreased with increase in test concentration but in the different chronic test concentrations, the overall time required for the completion of zoeal development did not differ significantly from that of control.

Keywords. *Clibanarius olivaceus*; chronic influence; zinc; survival rate; mean days of moulting.

1. Introduction

On both the sides of Vellar estuary (lat. 11°29′ N; long. 74°46′ E), cultivation of paddy is extensively practiced. To increase the yield, different types of pesticides are used which are ultimately drained into the estuary. These chemicals are non-degradable in nature and are persistent in the environment and can affect the larva, juvenile and adult of both endemic and migratory organisms in the estuary. Most of the pesticides in use contain heavy metals like zinc in their composition (organophosphorous and carbamates). Sewage released into the estuary from Portonovo town also contains zinc (Kumaraguru 1980). The present study aims to bring out the effect of this heavy metal on the larval stages of the most abundant hermit crab *Clibanarius olivaceus*.

2. Materials and methods

C. olivaceus for toxicological study was collected from the Vellar estuary. Ovigerous females were separated, offered new shells and kept in aquarium containing filtered sea water until hatching of larvae occurred. As soon as the larvae were liberated by the ovigerous crabs, they were separated in clean 500 ml beakers containing filtered sea water. The salinity of the sea water used in the present study was 35 ± 1% and the water temperature during the larval rearing period was 29 ± 1°C. 4·43 g of zinc sulphate ($ZnSO_4$ $5H_2O$—analar grade) dissolved in 1 litre of deionized water was used as the stock solution and the strength of this solution was 1 ppt. Serial dilutions were made to get required test concentrations.

2.1 *Test procedure*

In order to find out the 96 h LC_{50} value, 30 larvae each were used in different concentrations of zinc following the method of Ahsanullah and Arnott (1978). The acute toxicity test was run in 11 concentrations (1, 5, 10, 30, 50, 70, 90, 100, 125, 150 and 200 ppb). During the 96 h experimental period, no mortality was observed upto 70 ppb and the mortality increased after that. The 96 h LC_{50} value for zinc was found to be 100 ppb (figure 1). Three replicate beakers each containing 10 larvae were used for each test concentration. All larvae for each test came from a single berried crab and were less than 12 h old at the initiation of an experiment. Test beakers contained 100 ml of test water and were not aerated. Larvae were transferred daily to clean beakers containing fresh medium by means of a pipette and were fed with freshly hatched *Artemia* nauplii. The criterion for determining death was the absence of movement, when the larvae were prodded. Dead larvae were removed on each observation. Presently the larval development was studied in 7 sublethal concentrations (1, 5, 10, 30, 50, 70 and 90) besides the 96 h median lethal concentration.

3. Results

3.1 *Larval survival rate*

The survival rate of *C. olivaceus* zoeal stages at different test concentrations of zinc is given in figure 2. The overall survival rate was 80% in control. Mortality occurred in all the zoeal stages except zoeal stage II.

Survival rate in other test concentrations was lower than in the control and as the concentration increased the survival rate decreased and the lowest survival rate (46·6%) was found in 100 ppb concentration. Mortality occurred in all the stages except zoeal stage II.

3.2 *Mean days of moulting*

The mean days of moulting of each zoeal stage in different test concentrations is given in table 1 and figure 3.

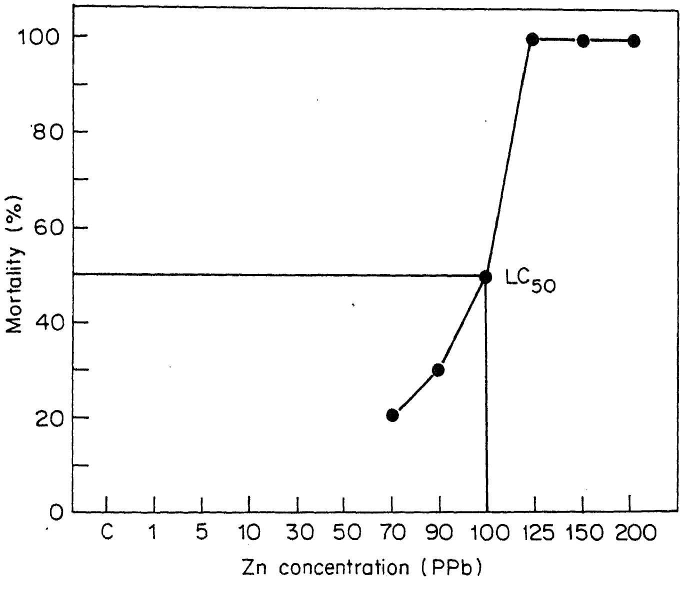

Figure 1. Toxicity of zinc on I zoea of *C. olivaceus* (96 h LC_{50}).

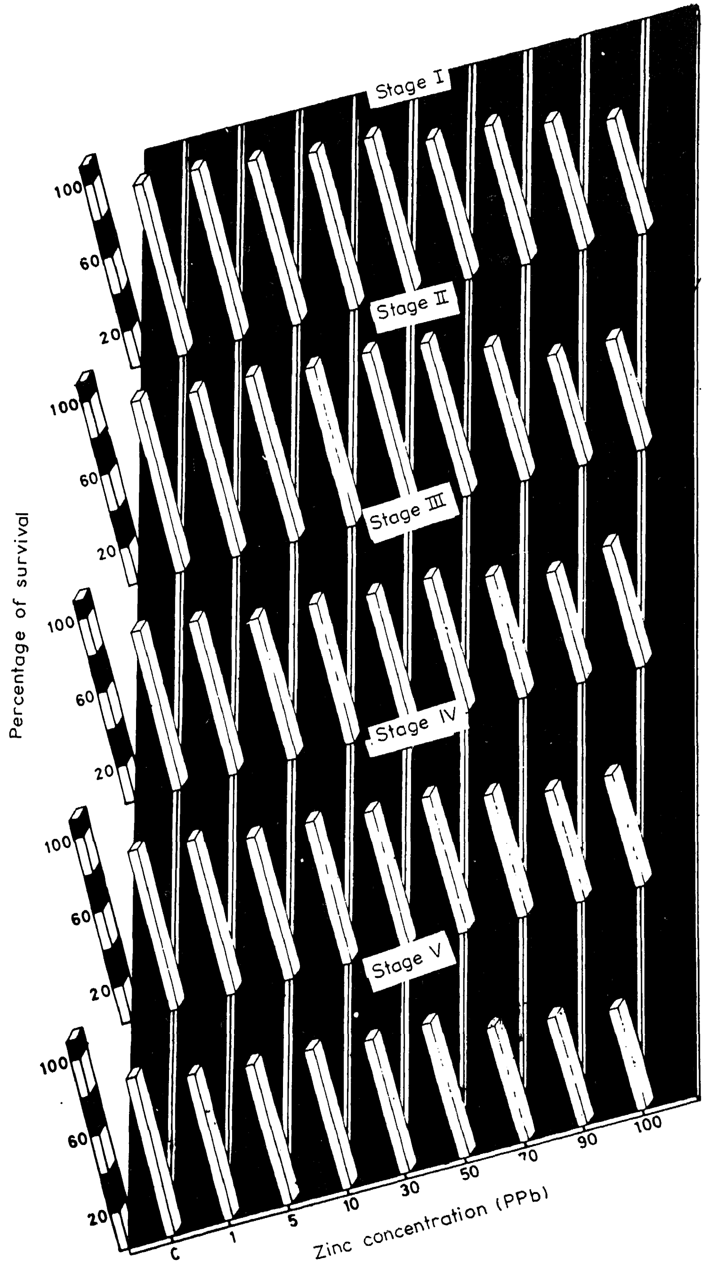

Figure 2. Average per cent survival of *C. olivaceus* zoeal stages I–V reared from hatching to glaucothoe in different concentrations of zinc.

3.3 *I Zoea* (*Carapace length* 0·9 *mm*)

Here, as the test concentration increased, intermoult duration also increased. Only in two test concentrations the intermoult duration was shorter than that of the control and in other concentrations the duration was more. The maximum of 10·37 days was

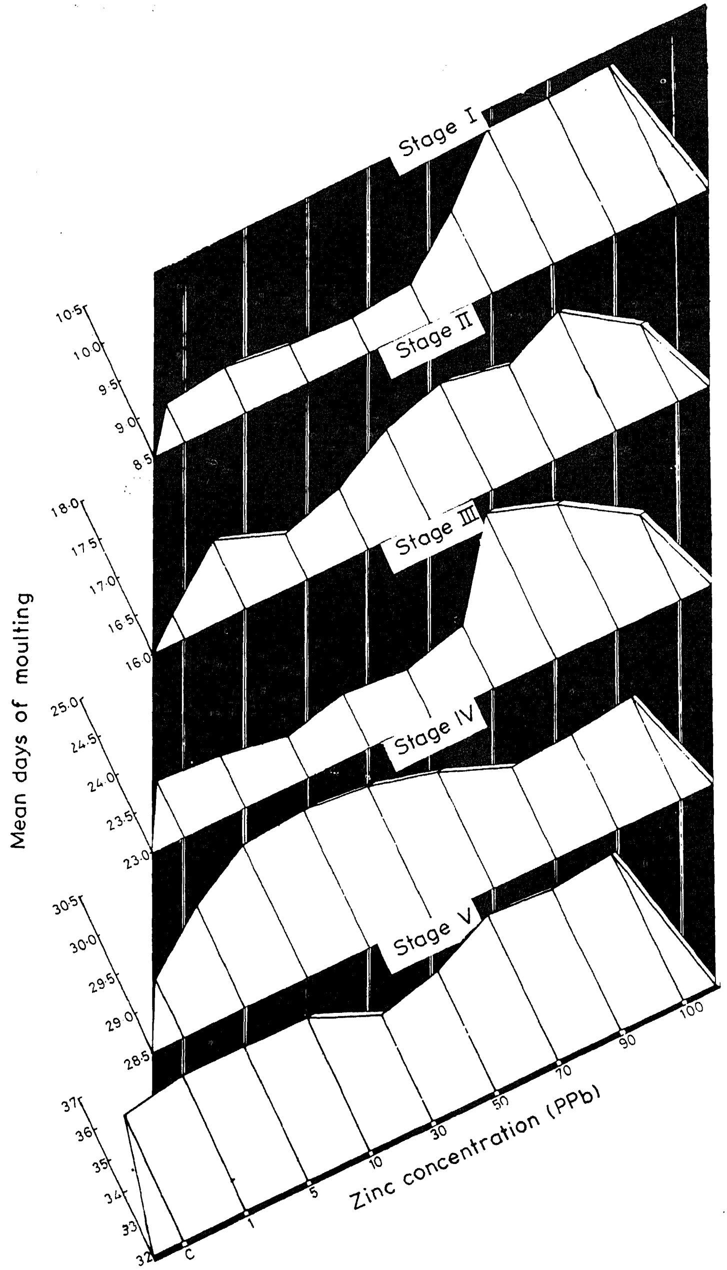

Figure 3. Mean days of moulting of zoeal stages I–V of *C. olivaceus* in different test concentrations of zinc.

encountered in 100 ppb concentration. *T* test was used to find out the differences in intermoult duration between different concentrations (table 2). Intermoult duration in higher concentrations differed significantly from that of control and other lower concentrations.

Table 1. Mean days of moulting of zoeal stages I–V of *C. olivaceus* (along with standard deviation and variance) exposed to different test concentrations of zinc.

Stage	Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
I Zoea Mean	9·04	9·11	9·00	8·96	9·12	9·61	10·30	10·33	10·37
	±	±	±	±	±	±	±	±	±
SD	1·00	0·83	0·88	0·82	0·86	0·99	0·82	0·73	0·68
Variance	1·00	0·69	0·77	0·68	0·75	0·98	0·88	0·53	0·47
Number	28	28	27	26	26	23	23	21	19
II Zoea Mean	16·32	16·88	16·59	16·81	17·19	17·44	17·23	17·56	17·00
	±	±	±	±	±	±	±	±	±
SD	1·06	1·59	1·62	1·58	0·75	0·92	0·97	1·25	1·19
Variance	1·12	2·53	2·62	2·50	0·56	0·84	0·95	1·56	1·42
Number	28	25	27	26	26	25	22	18	18
III Zoea Mean	23·75	23·68	23·5	23·70	23·64	23·82	24·90	24·61	24·06
	±	±	±	±	±	±	±	±	±
SD	1·37	1·38	1·38	1·40	1·50	1·53	1·52	1·33	1·30
Variance	1·88	1·90	1·90	1·96	2·25	2·34	2·31	1·77	1·69
Number	26	25	23	23	22	22	20	18	18
IV Zoea Mean	29·24	29·83	30·23	30·27	30·19	29·95	29·67	29·71	29·81
	±	±	±	±	±	±	±	±	±
SD	1·69	1·64	1·48	1·58	1·57	1·50	1·50	1·65	1·56
Variance	2·86	2·69	2·19	2·50	2·46	2·25	2·25	2·72	2·43
Number	25	23	22	22	21	21	18	17	16
V Zoea Mean	36·00	36·30	36·24	36·24	35·38	35·80	36·65	36·50	36·71
	±	±	±	±	±	±	±	±	±
SD	1·85	1·92	1·73	1·89	1·86	2·24	2·18	2·16	2·23
Variance	3·39	3·69	2·99	3·57	3·46	5·02	4·75	4·67	4·97
Number	24	23	21	21	21	20	17	16	14

Table 2. *T* values for the differences in mean intermoult duration of I zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	0·2849	0·1574	0·2701	0·2755	2·0346[a]	4·8384[d]	5·1013[d]	5·1040[d]
1 ppb		0·4775	0·6590	0·0432	1·9617	5·1072[d]	5·3590[d]	5·4635[d]
5 ppb			0·1707	0·5008	2·3097[b]	5·3667[d]	5·6026[d]	5·6866[d]
10 ppb				0·6822	2·5069[b]	5·6770[d]	5·9623[d]	6·0712[d]
30 ppb					1·8483	4·8674[d]	4·7957[d]	5·2063[d]
50 ppb						2·5683[c]	2·7261[c]	2·8297[c]
70 ppb							0·1274	0·2950
90 ppb								0·1783
100 ppb								

[a]$P<0·05$; [b]$P<0·02$; [c]$P<0·01$; [d]$P<0·001$.

Table 3. *T* values for the differences in mean intermoult duration of II zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	1·5239	0·7347	1·3471	3·4631[c]	4·1613[c]	3·0547[c]	3·6137[c]	2·0246[a]
1 ppb		0·6508	1·5750	0·8962	1·4771	0·8941	1·5079	0·2697
5 ppb			0·5003	1·7208	2·3047[b]	1·6278	2·1487[a]	0·9198
10 ppb				1·1076	1·6595	1·0829	1·6801	0·4314
30 ppb					1·0505	0·1607	1·6302	0·6502
50 ppb						0·7609	0·4142	1·3693
70 ppb							0·9797	0·6718
90 ppb								1·3763
100 ppb								

[a]$P<0·05$; [b]$P<0·02$; [c]$P<0·001$.

3.4 *II Zoea* (*Carapace length* 1·08 *mm*)

The mean days of moulting for control was 16·32 days. This duration increased as the test concentration increased and the highest (17·56 days) was in 90 ppb concentration. However in the highest concentration of 100 ppb there was a fall and the mean days of moulting was lower than in 30, 50, 70 and 90 ppb concentrations. Mean days of moulting in higher concentrations (30, 50, 70, 90 and 100 ppb) differed significantly from that of control, and the difference between these concentrations was not statistically significant (table 3).

3.5 *III Zoea* (*Carapace length* 1·20 *mm*)

Trend in mean days of moulting in this stage differed from that of the previous two zoeal stages. With increase in test concentration upto 30 ppb, the mean day of moulting declined from that of control. But in 50 and 70 ppb concentrations it increased slightly and again in 90 and 100 ppb concentrations there was a decline but mean days of moulting in these two concentrations was more than that of control.

Mean days of moulting in 70 and 90 ppb concentrations differed significantly from that of control (table 4).

3.6 *IV Zoea (Carapace length* 1·73 *mm)*

Trend in mean days of moulting resembled more or less that of the previous zoeal stage. It increased from control upto 10 ppb concentration and then declined in 30, 50 and 70 ppb concentrations and again increased slightly in 90 and 100 ppb concentrations. The lowest was recorded in control (29·24 days) and the highest (30·27 days) in 10 ppb concentration. Mean days of moulting of only 5 and 10 ppb concentrations differed significantly from that of control (table 5).

3.7 *V Zoea (Carapace length* 2·19 *mm)*

As the test concentrations increased mean days of moulting also increased and declined. Shortest duration (35·38 days) was encountered in 30 ppb concentration and the highest (36·71 days) in the highest concentration. *T* values did not suggest significant difference between control and test concentrations and among test concentrations (table 6).

Table 4. *T* values for the differences in mean intermoult duration of III zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	0·1817	0·5845	0·1261	0·2652	0·2865	2·7804[c]	2·0702[a]	0·6774
1 ppb		0·4016	0·0498	0·0952	0·3300	2·8188[c]	2·2142[b]	0·9129
5 ppb			0·4393	0·2796	0·6917	3·1221[d]	2·5511[c]	1·2759
10 ppb				0·2746	0·5617	2·7250[c]	2·1104[a]	0·8427
30 ppb					0·3940	2·7017[c]	2·1900[a]	0·9346
50 ppb						2·2921[b]	1·7214	0·8253
70 ppb							0·6226	1·8203
90 ppb								1·2544
100 ppb								

[a]$P<0{\cdot}05$; [b]$P<0{\cdot}02$; [c]$P<0{\cdot}01$; [d]$P<0{\cdot}001$.

Table 5. *T* values for the differences in mean intermoult duration of IV zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	0·5466	0·4486	0·4309	1·1215	0·3251	1·0319	0·7848	1·0614
1 ppb		1·1084	0·1045	1·6108	0·7880	0·5380	0·3038	0·5926
5 ppb			1·7891	1·2824	0·7059	0·6470	0·4067	0·7015
10 ppb				1·4863	0·6809	0·6207	0·3897	0·7609
30 ppb					0·6543	1·9326	1·6920	1·9142
50 ppb						1·1644	0·9461	0·8983
70 ppb							0·1984	0·0755
90 ppb								0·2616
100 ppb								

Table 6. *T* values for the differences in mean intermoult duration of V zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	1·2249	2·1218[a]	2·1474[a]	1·9611	0·9287	0·8615	0·9932	1·0846
1 ppb		0·8576	0·9154	0·7424	0·2523	0·3216	0·2281	0·0382
5 ppb			0·0866	0·8694	0·6160	1·1834	1·0353	0·8447
10 ppb				0·1498	0·6801	1·2216	1·0764	0·8906
30 ppb					0·5067	1·0530	0·9170	0·7320
50 ppb						0·5811	0·4220	0·2765
70 ppb							0·9214	0·6918
90 ppb								1·7870
100 ppb								

[a] $P < 0·05$.

4. Discussion

Acute and sublethal concentrations of pollutants are defined by Epifanio (1979). Acute concentrations are those, in which less than 10% of the larvae reach the crab stage, whereas sublethal concentrations are those, in which there is significant mortality or delay in moult, but more than 10% of the larvae reach the crab stage. However, these definitions are valid, when survival to the crab stage is high in the control but not when the survival is low (Bookhout and Costlow 1975). Presently the overall survival rate of larvae in control was more and also in different concentrations of bioassay used the survival rate was more than 10%. Thus the sublethal concentrations of the present study perfectly fit in with the definition of Epifanio (1979).

4.1 *Survival rate*

In the present study, larvae of *C. olivaceus* were reared in 7 sublethal and 96 h LC_{50} level concentrations from hatching to glaucothoe stage. With increase in test concentrations of zinc survival rate of larvae decreased. Similar findings have been reported elsewhere also. Epifanio (1971) studied the effect of dieldrin (organochloride pesticide) on the larval development of the crabs *Leptodius floridanus* and *Panopeus herbstii* and found that as the test concentrations increased, the survival rate of larvae decreased. Christiansen *et al* (1977) studied the effect of hydroprene on the larval development of mud crab *Rhithropanopeus harrisii* and found that as the test concentrations increased, the number of larvae reaching the megalopa stage decreased. The study of Bookhout *et al* (1976) about the effect of methoxychlor on larval development of mud crab and blue crab *R. harrisii* and *Callinectes sapidus* also showed similar results. They also noted that the sharpest reduction in survival took place in the megalopa stage and they attributed that this was possibly due to transformation of zoeal tissues to adult tissues. During this period the pesticides, which had been stored in fat during zoeal development were released into the blood stream and this caused mortality in some unknown way. Presently the survival rate during post larval stage (glaucothoe stage) was not found, as these hermit crab larvae require minute shells for occupation before moulting into the first crab instar (Reese

1963). But in another species of crab *R. harrisii*, megalopa stage was less sensitive to hydroprene than were the zoeal stages and thus different species of crabs show differential sensitivity to the same pesticide at different stages of development (Christiansen *et al* 1977). Moreover to compare the sensitivity of different zoeal stages, it will be better if each zoeal stage is introduced to the test medium freshly. The above observations including the present one show the prolonged influence of test concentrations during development.

4.2 *Mean days of moulting*

Presently, mean days of moulting under different test concentrations of heavy metal, zinc, showed interesting results. With increase in test concentration up to 10 ppb, the mean day of moulting also increased. In 30 and 50 ppb concentrations it was found to be less than that of control. Again it was more in the highest concentrations of 70, 90 and 100 ppb. But statistically the overall time taken for completion of zoeal development in different test concentrations did not differ significantly from that of control. Buchanan *et al* (1970) observed delay in the moulting of larvae reared in sublethal concentrations of sevin from zoeal stages I to II. Similar results have been reported for the zoeal stages of *R. harrisii*, *Menippe mercenaria* and *C. sapidus* by Bookhout *et al* (1972, 1976) caused by different pollutants.

Stress in higher concentrations of pollutant may act in two different ways. First an alteration in normal development i.e., an increase in number of zoeal stages. Bookhout *et al* (1972) found the mirex to interpose an additional zoeal stage (VI zoea) in the normal development of 5 zoeal stages. As most of the crabs consistently have a definite number of zoeal stages perhaps both larval moulting and rate of development are controlled by the same mechanism. There are two exceptions to the above and those are the larvae of *C. sapidus* and *M. merceneria*. Costlow (1968) suggested that atleast two mechanisms may be involved—one that controls moulting and a second normally synchronized with first that regulates the rate of morphological development in larval stages. The site for the regulation of morphological development appears situated in the larval eyestalk for when both eyestalks of *R. harrisii* were removed the larvae passed through an extra zoeal stage without morphological development. Secondly stress in higher concentrations of pollutants may exert an action on the mechanism, which regulates morphological and moulting changes thus speedy development of larvae (Bookhout *et al* 1972). Toxicity studies of pesticides on crab larvae support the above view. Many authors suggested that toxicity of pesticides like sevin was associated with the moulting process (Epifanio 1979). Moulting in crab larvae is known to be a neuroendocrine function (Costlow 1963, 1966a, b) and naturally sublethal toxicity of neurotoxins might be expected to interfere, with moulting process. Just like pesticides sevin, hydroprene and dieldrin heavy metal zinc may also be a neurotoxin and it may also interfere with moulting. Presently in some sublethal concentrations of zinc, the mean days of moulting was found to be shorter.

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In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure

M SRINIVASULU REDDY and K V RAMANA RAO
Department of Marine Zoology, SV University PG Centre, Kavali 524 202, India

Abstract. Penaeid prawn, *Penaeus indicus* (H Milne Edwards), was subjected to acute (2 day) and chronic (15 day) exposure to sublethal concentration (0·2 ppm) of phosphamidon, and changes in the carbohydrate metabolism of phasic and tonic muscles were investigated. Differential patterns in carbohydrate metabolism were witnessed. The glycogen content of phasic muscle was elevated, whereas that of tonic muscle was depleted in response to acute exposure. During chronic exposure, both muscle tissues had elevated glycogen content. In general, both phasic and tonic muscles exhibited low-level of glycolysis and elevated oxidative pathway operation, leading to elevated glycogen content. Both, phasic and tonic muscle metabolisms were oriented towards conservation of carbohydrates and lesser production of organic acids during chronic exposure, as a possible adaptive metabolic mechanism of this prawn enabling it to counteract the toxic stress imposed by phosphamidon.

Keywords. *Penaeus indicus*; phosphamidon; carbohydrate metabolism; glycolysis.

1. Introduction

Despite concern over environmental contamination by pesticides, man continues to depend on the use of pest-control chemicals for increase in agricultural productivity and disease control. Increased use of organophosphate (OP) pesticides due to suspended or cancelled registration of chlorinated hydrocarbon pesticides, generates a need for information on their effects on the aquatic environment (Bookhout and Monroe 1977; Nimmo 1979). OP pesticides may enter surface and estuarine water through industrial effluent, run off, or direct application (Coppage and Matthews 1974). OP insecticides are believed to be potent inhibitors of the enzyme acetylcholinesterase (AchE), which modulates the neurotransmittor substance, acetylcholine, at the nerve cell junctions, i.e., synapses. Inhibition of AchE by OP insecticides and also by their metabolites causes accumulation of acetylcholine and disruption of normal neurotransmission (O' Brien 1967) which will further lead to some physiological and metabolic changes in several non-target organisms, including a variety of crustaceans (Couch 1979). Phosphamidon, like many other OP insecticides, is widely used to control crop pests presumably because it is biodegradable and leaves residues, but for a short time. Eventhough phosphamidon decomposes rapidly in the environment, it may be toxic to target and non-target organisms alike. Since it is relatively a non-persistent insecticide, repeated applications may be necessary for control of pests which may result in cumulative reduction of AchE. Hence, increasing concentrations of phosphamidon have to be used to control pests and the danger to non-target organisms becomes greater (Nagabhushanam *et al* 1982; Srinivasulu Reddy *et al* 1985a, b). The present investigation is aimed at understanding the toxic impact of phosphamidon in phasic and tonic muscle carbohydrate metabolism of a penaeid prawn, *Penaeus indicus* (H Milne Edwards), after acute and chronic exposure to a sublethal concentration. This prawn is considered to be a sensitive indicator of marine and estuarine pollution (Butler 1966) and also forms one of the commercially important fishery of India.

2. Materials and methods

P. indicus (10 ± 1 g) were collected from Buckingham canal, Thummalapenta seacoast, near Kavali. They were kept in large aquaria with continuous aeration to acclimatize to laboratory condition for 1 week under constant salinity 15 ± 1‰, pH 7·2 ± 0·1 and temperature 23 ± 2°C. They were fed *ad libitum* with oil cake powder. Technical grade phosphamidon [92% w/v; 0, 0–dimethyl–0–(1-methyl–2–chloro–2–diethyl–carbomoyl–vinyl) phosphate] obtained from CIBA-GEIGY, Bombay was used as test chemical. The preparation of test solution was described earlier (Srinivasulu Reddy *et al* 1985a). LC_{50} value (computed as per Finney 1964) was found to be 1·2 ppm for 48 h. The laboratory-acclimated intermoult prawns were divided into 3 groups viz control and acutely and chronically exposed. The control prawns were maintained in seawater without test chemical. Prawns were exposed to 0·2 ppm for acute (2 day) and for chronic (15 day) exposure, respectively. After 50 prawns were sacrificed, phasic and tonic muscles were isolated and rapidly chilled by placing them in cold crustacean ringer. These tissues were used for biochemical analysis.

Glycogen (Carrol *et al* 1956), pyruvic acid (Friedmann and Hangen 1942), and lactic acid (Barker and Summerson 1941, modified by Huckabee 1956) were estimated in phasic and tonic muscles of control and phosphamidon exposed (PE) prawns. The activities of phospharylase '*a*' and '*ab*' were estimated in the direction of glycogen synthesis (Cori *et al* 1955). Homogenate (5%) was prepared in an aqueous medium containing 0·037 on ethylene diamine tetraacetic acid (EDTA) and 0·1 M sodium fluoride, pH 6·5, as recommended by Guillary and Mammaerts (1962). The homogenate was centrifuged for 15 min at 2500 rpm and the supernatant was diluted 4 times with cysteine (0·03 M), B-glycerophosphate (0·015 M) buffer, pH 6·5. The diluted enzyme (0·4 ml) was added to 0·2 ml of 2% glycogen and incubated for 20 min at 37°C. The reaction was started by the addition of 0·2 ml of 0·016 M glucose-1-phosphate (G-1-P) to one tube (Phosphorylase '*a*'), 0·2 ml of G-1-P and 0·004 M adenosine-5-monophosphate to the other (phosphorylase '*ab*'). After incubation for 15 min for phosphorylase '*ab*' (total) and 30 min for phosphorylase '*a*' (active) activities, the reaction was stopped by the addition of 10% sulphuric acid. The inorganic phosphate (Pi) liberated was estimated by the method of Fiske and Subba Row (1925) and Protein by the method of Lowry *et al* (1951). Aldolase activity was estimated by the method of Bruns and Bergmayer (1965). The activity levels of succinate dehydrogenase (SDH), NAD-glutamate dehydrogenase (GDH), and NAD-lactate dehydrogenase (LDH) were estimated by the method Nachlas *et al* (1960). The activity level of NADP glucose-6-phosphate dehydrogenase (G-6-PD) was estimated by the method of Georg and Waller (1965). The statistical correlations were conducted using Students '*t*' test as described by Bailey (1965).

3. Results and discussion

The data presented in tables 1 and 2 illustrate the metabolic variations in phasic and tonic muscles of prawn subjected to acute exposure to a sublethal concentration of phosphamidon. With acute exposure, glycogen content was depleted (23%) in tonic muscle; phasic muscle had (28%) elevation in glycogen content. Phosphorylase '*ab*' was depleted in both phasic (26%) and tonic (45%) muscles. Phosphorylase '*a*' was inhibited (28%) in tonic muscle, with no significant change (3%) in phasic muscle. Phosphorylase '*b*' was depleted by 47% and 39% in tonic and phasic muscles, respectively. Aldolase activity was depleted in phasic (23%) and tonic (52%) muscle, whereas NAD–LDH was elevated by 36% in phasic and 50% in tonic muscles. In tonic muscle, pyruvic acid content was elevated by 24% and lactic acid content showed no significant change. Phasic muscle showed no significant change in pyruvic acid content, whereas lactic acid content was depleted (27%). Glucose-6-phosphate dehydrogenase (G-6-PDH) activity levels were decreased by 12% in tonic muscle and 23% in phasic muscle. GDH activity was elevated in both the muscles. Both SDH and malate dehydrogenase (MDH) activities were elevated in tonic and phasic muscles after acute exposure to phosphamidon.

The tonic and phasic muscles of chronically-exposed prawns (tables 3 and 4) showed significant elevation in the glycogen content, the maximum being in phasic muscle (64%). The activity levels of Phosphorylase '*ab*' and '*b*' were significantly depleted in both the muscles, but phosphorylase '*a*' was significantly elevated. Aldolase activity was inhibited by 70% in tonic muscle, but was elevated by 28% in phasic muscle. The levels of pyruvic acid and lactic acids were decreased in both PE

Table 1. Levels of glycogen and lactic acid and the activity levels of phosphorylase and aldolase in tonic and phasic muscles of control prawns and those acutely exposed to phosphamidon.

Parameter	Control	Tonic muscle	Control	Phasic muscle
Glycogen[1]	6·14 ± 0·38	4·71 ± 0·22 (−23)	10·18 ± 0·65	13·03 ± 0·68 (+28)
Phosphorylase '*a*'[2]	3·12 ± 0·18	2·25 ± 0·11 (−28)	3·84 ± 0·25	3·71^a ± 0·22 (−3)
Phosphorylase '*ab*'[2]	10·85 ± 0·46	5·91 ± 0·22 (−46)	12·05 ± 0·65	8·86 ± 0·51 (−26)
Phosphorylase '*b*'[2]	6·92 ± 0·38	3·68 ± 0·21 (−47)	7·78 ± 0·51	4·72 ± 0·34 (−39)
Aldolase[3]	3·89 ± 0·23	1·91 ± 0·10 (−51)	4·13 ± 0·27	3·19 ± 0·20 (−23)
Pyruvic acid[4]	5·94 ± 0·48	7·35 ± 0·49 (+24)	6·25 ± 0·56	5·83^a ± 0·39 (−7)
Lactic acid[5]	1·33 ± 0·21	1·49^a ± 0·10 (+12)	1·41 ± 0·20	1·03 ± 0·11 (−27)

Each value is mean of 6 individual observations.
Mean ± SD; + and − indicates per cent increase or decrease over control.
All values are statistically significant at $P < 0{\cdot}001$ except *a*: Not significant.
Values are expressed in 1: mg/gm wet tissue; 2: µ mol pi formed/mg protein/h; 3: µ mol FDP cleaved/mg protein/h; 4: µ mol/g wet tissue; 5: mg/g wet tissue.

Table 2. Levels of glycogen and lactic acid and the activity levels of phosphorylase and aldolase in tonic and phasic muscles of control prawns and those chronically exposed to phosphamidon.

Parameter	Control	Tonic muscle	Control	Phasic muscle
Glycogen[1]	6·23 ± 0·29	9·84 ± 0·31 (+58)	10·32 ± 0·71	16·32 ± 0·83 (+64)
Phosphorylase '*a*'[2]	3·28 ± 0·21	3·91 ± 0·17 (+19)	3·91 ± 0·28	4·69 ± 0·33 (+20)
Phosphorylase '*ab*'[2]	11·21 ± 0·49	4·02 ± 0·13 (−64)	12·71 ± 0·63	3·92 ± 0·26 (−69)
Phosphorylase '*b*'[2]	7·09 ± 0·28	1·85 ± 0·10 (−74)	8·03 ± 0·58	1·44 ± 0·12 (−82)
Aldolase[3]	4·08 ± 0·21	1·22 ± 0·11 (−70)	4·45 ± 0·31	5·69 ± 0·38 (+28)
Phravic acid[4]	6·18 ± 0·31	4·50 ± 0·18 (−27)	6·89 ± 0·33	3·51 ± 0·22 (−49)
Lactic acid[5]	1·45 ± 0·20	1·08 ± 0·09 (−26)	1·52 ± 0·23	0·72 ± 0·08 (−53)

Each value is mean of 6 individual observations.
Mean ± SD; + and − indicates per cent increase or decrease over control.
All values are statistically significant at $P < 0·001$.
Values are expressed in 1: mg/g wet tissue; 2: μ mol pi formed/mg protein/h; 3: μ mol FDP cleaved/mg protein/h; 4: μ mol/g wet tissue; 5: mg/g wet tissue.

Table 3. Activity levels of G-6-PDH, NAD-LDH, GDH, SDH and MDH in tonic and phasic muscles of control prawns and those acutely exposed to phosphamidon.

Enzyme	Control	Tonic muscle	Control	Phasic muscle
G-6-PDH	1·31 ± 0·15	1·15[a] ± 0·10 (−12)	1·40 ± 0·19	1·08 ± 0·09 (−23)
LDH	0·103 ± 0·019	0·155 ± 0·010 (+50)	0·121 ± 0·012	0·165 ± 0·019 (+36)
GDH	0·651 ± 0·049	1·080 ± 0·105 (+66)	0·784 ± 0·056	1·120 ± 0·130 (+43)
SDH	0·375 ± 0·032	0·465 ± 0·041 (+24)	0·856 ± 0·069	0·992[b] ± 0·071 (+16)
MDH	0·068 ± 0·007	0·092 ± 0·005 (+35)	0·135 ± 0·011	0·195 ± 0·014 (+44)
SDH : GDH	0·576	0·431 (−25)	1·09	0·886 (−19)
SDH : LDH	3·641	2·994 (−18)	7·07	6·01 (−15)

Each value is mean of 6 individual observations.
Mean ± SD: + and − indicate per cent increase or decrease over control.
All values are statistically significant at $P < 0·001$ except *a*: $P < 0·02$; *b*: $P < 0·01$.
Values are expressed in μ mol formazan formed/mg protein/h.

Table 4. Activity levels of G-6-PDH, NAD-LDH, GDH, SDH and MDH in tonic and phasic muscles of control prawns and those chronically exposed to phosphamidon.

Enzyme	Control	Tonic muscle	Control	Phasic muscle
G-6-PDH	1·43 ± 0·12	2·32 ± 0·25 (+62)	1·45 ± 0·21	2·28 ± 0·32 (+57)
LDH	0·114 ± 0·007	0·210 ± 0·015 (+84)	0·128 ± 0·014	0·244 ± 0·029 (+91)
GDH	0·662 ± 0·031	1·220 ± 0·109 (+84)	0·803 ± 0·049	1·630 ± 0·321 (+103)
SDH	0·389 ± 0·038	0·595 ± 0·049 (+53)	0·889 ± 0·061	1·190 ± 0·305 (+34)
MDH	0·075 ± 0·006	0·137 ± 0·018 (+83)	0·149 ± 0·014	0·242 ± 0·028 (+62)
SDH:GDH	0·588	0·113 (−81)	1·10	0·73 (−34)
SDH:LDH	3·412	2·833 (−17)	6·94	4·88 (−30)

Each value is mean of 6 individual observations.
Mean ± SD: + and − indicates per cent increase or decrease over control.
All values are statistically significant at $P < 0{\cdot}001$.
Values are expressed in μ mol formazan formed/mg protein/h.

prawn muscles. NAD–LDH and G-6-PDH activity levels were significantly elevated in both PE prawn muscles. The activity levels of GDH, SDH and MDH were significantly elevated in both PE prawn muscles after chronic exposure.

Phasic and tonic muscles of *P. indicus*, exhibited differential patterns of carbohydrate metabolism following exposure both acutely and chronically to a sublethal concentration of phosphamidon. The glycogen content might have been actively mobilized towards the haemolymph glucose under this pesticide stress to provide maximum energy, similar to observations in different crustacean groups applied to other environmental stress conditions (Bhagyalakshmi *et al* 1983, 1984). Phosphamidon-induced stress may lead to depleted glycogen levels in tonic muscle on acute exposure to phosphamidon. Decreased activities of phosphorylase '*ab*' (total), '*a*' (active) and '*b*' (inactive) in tonic muscle were suggestive of regulation imposed at the metabolic levels to prevent further glycogen degradation. Depletion of aldolase activity was also suggestive of a decreased rate of glycolysis in this tonic muscle. However, the pyruvic acid level in tonic muscle tissues increased in prawns acutely exposed to phosphamidon. The elevated activity level of NAD–LDH, observed in the present study might be responsible for the increased pyruvic acid content (Srinivasulu Reddy *et al* 1985c). Increased activity of alanine aminotransferase (AlAT) in the muscle exposed to phosphamidon, however might also suggest mobility of amino acids towards the formation of pyruvic acid (Srinivasulu Reddy *et al* 1985d). Significantly lower G-6-PDH activity may be attributed to decreased mobilization of glycogen into the hexose monophosphate (HMP) pathway. Activity levels of SDH and MDH were elevated, suggesting stepped up oxidative metabolism in tonic muscle tissues of pesticide exposed prawn. This elevation in the oxidative phase might be due to mobilization of amino acids as revealed by elevated GDH and pyruvic acid and as indicated by increased LDH

(Bhagyalakshmi *et al* 1984). SDH:GDH and SDH:LDH ratios were decreased in these muscles indicating that the extent of elevation in SDH did not correlate with elevation of either GDH or LDH, suggesting the possibility of diversion of citric acid intermediates (Huggins and Munday 1968). Hence, in response to acute exposure to a sublethal concentration of phosphamidon, the tonic muscle had inhibited glycolysis with depleted glycogen content.

Phasic muscle however, showed a different trend in that it had a significant elevation in the glycogen content on a acute exposure to phosphamidon. Similar kind of observations were also made by Sreenivasulu Reddy *et al* (1983) in *Oziotelphusa senex senex* during chronic sumithion exposure. Since NAD–LDH and GDH activities were significantly elevated, active mobilization of lactic acid and amino acids into the oxidative metabolism can be envisaged. Phasic muscle metabolism was also oriented towards inhibited glycolysis (Hochachka *et al* 1962) as indicated by decreased aldolase activity leading to elevated glycogen content, (Sreenivasulu Reddy *et al* 1983). In spite of the operation of similar metabolic events in tonic and phasic muscle, the phasic muscle had elevated glycogen content, which might be due to participation of tissue lipid components in metabolism (Huggins 1966; Bhagyalakshmi 1981). The free fatty acid content was depleted in the muscle suggesting the participation of free fatty acids in tissue oxidations. Such a possibility of fatty acid oxidations might be responsible for the suppressed glycolysis leading to elevation in glycogen content (Bhagyalakshmi 1981; Chang and O' Connor 1983).

Muscle metabolism seems to be different in acutely and chronically-exposed prawn, since with chronic exposure to phosphamidon both tonic and phasic muscles had elevated glycogen content with the maximum increase in phasic muscle. Most probably phasic muscle forms a reserve site for nutrients and its increased glycogen content suggests storage of glycogen reserves for general muscular activity (Hohnke and Scheer 1970). In tonic muscle, inhibited aldolase activity in the presence of glycogen suggests metabolic shifts towards pathways other than glycolysis (Dean and Vernberg 1965). Since the activity level of G-6-PDH was considerably elevated, the possible mobilization of glycogen and glucose towards the hexose monophosphate pathway can be visualized. This might be an adaptation toward decreasing formation of lactic and pyruvic acid, aiming for decreased acid production in the phasic and tonic muscles. Hence this suggests tissue compensation at the metabolic level in response to the stress imposed by phosphamidon exposure. Elevated activity levels of LDH and GDH were suggestive of increased mobilization of lactic acid and amino acids into the oxidative metabolism. Consequent on such changes, there were not only depleted lactic acid levels in PE prawn muscles, but also an elevated oxidative phase of metabolism as indicated by SDH and MDH activities. Metabolism of phasic muscle was oriented towards mobilizing glycogen into hexosemonophosphate shunt pathway, since phosphorylase, aldolase and G-6-PDH activities are elevated. Hence both phasic and tonic muscles in chronically exposed prawns have regulated the carbohydrate metabolism towards decreasing the production of metabolic acids.

From the present investigation it can be concluded that during chronic exposure of penaeid prawn to a sublethal concentration of phosphamidon, phasic and tonic muscle metabolism was oriented toward lower production of metabolic acids and increased carbohydrate reserves with switching over to aerobic phase. These modifications in tissue metabolism might represent compensatory mechanisms leading to adaptive changes which provide positive survival chances in phosphamidon-polluted aquatic environments.

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Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure

M SRINIVASULU REDDY and K V RAMANA RAO
Division of Toxicology, Department of Marine Zoology, S V University P G Centre, Kavali 524 202, India

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Abstract. Changes in the nitrogen metabolic profiles of midgut gland, muscle and gill tissues were studied, following the exposure of *Penaeus indicus* to acute (2 day) and chronic (10, 20 and 30 day) exposure to sublethal concentration (0·2 ppm) of phosphamidon. The proteolytic activity of phosphamidon exposed prawn tissues was significantly elevated. All the phosphamidon exposed prawn tissues showed efficient mechanisms of ammonia detoxification in the chronically exposed prawns when compared to acutely exposed prawns. The survivability of these prawns in the polluted habitats might be due to the operation of compensatory adaptive mechanisms in the metabolic profiles such as increased biosynthesis of different kinds of proteins and also the detoxification and transformation of ammonia, the toxic nitrogenous end product of aquatic organisms.

Keywords. Phosphamidon; protease; glutamate dehydrogenase; *Penaeus indicus*.

1. Introduction

Aquatic pollution has been increasing at an alarming rate due to indiscriminate use of various types of pesticides (Matsumura *et al* 1972; Holden 1973). Today most of the alterations in the natural environment are contributed by the widespread usage of different formulations of organophosphorous (OP) insecticides when compared to the persistent and highly toxic organochlorine insecticides to several nontarget organisms (Butler 1966; Bookhout and Monroe 1977). Eventhough the OP insecticides are considered to be nonpersistent, they inhibit acetylcholinesterase (AChE) activity with a subsequent disruption in the nerve impulse transmission (O'Brien 1967) and which will further lead to some physiological and metabolic changes of several nontarget organisms, which include a variety of crustaceans (Bhagyalakshmi 1980; Avelin Mary 1984; Srinivasulu Reddy *et al* 1985a, b). Phosphamidon, an OP insecticide has a wide range of applications in agriculture as an effective systemic pesticide. The present investigation deals with the toxic impact of sublethal concentration of phosphamidon on the tissue nitrogen metabolism of a penaeid prawn *Penaeus indicus* during acute (2 day) and chronic (10, 20 and 30 day) exposure periods.

2. Material and methods

Penaeid prawns *P. indicus* (H Milne Edwards) were collected from the Buckingham canal near Thummalapenta seacoast, Kavali. Only intermolt uninjured prawns of the size 75 ± 5 mm and weight 2·5 ± 0·5 g were selected and acclimatized to laboratory for 1 week at constant salinity of 15 ± 1‰, pH 7·1 ± 0·2 and temperature 23 ± 2°C.

They were fed *ad lib*, diet of oilcake powder. The media in which they were placed was changed for every 48 h.

Technical grade phosphamidon (92% w/v) (0, 0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) obtained from CIBA-GEIGY, Bombay was used as test chemical. A stock solution of 1000 ppm phosphamidon (1 mg in 1 ml) was prepared and appropriate working concentrations were prepared by dilution with seawater. The concentrations selected ranged from 0·2–1·5 ppm with a difference of 0·2 ppm. LC_{50} value was found to be 0·9 ppm for 48 h. Laboratory acclimatized prawns were exposed to a sublethal concentration of 0·2 ppm as described earlier (Srinivasulu Reddy *et al* 1985a). In the present study, the sublethal concentration was selected as it closely approximates the levels found in the environment (Nimmo 1979). The prawns were divided into 5 batches viz control and acutely exposed (2 day exposed) and chronically exposed (10, 20 and 30 day exposed) groups. Equal number of prawns were kept untreated for the same period under similar conditions served as controls. Prawns (50) were sacrificed from each glass aquarium i.e., after 2, 10, 20 and 30 days of phosphamidon exposure. The midgut gland, muscle and gill tissues were isolated, rinsed in crustacean ringer. The protein content was estimated by Lowry *et al* (1951) using bovine serum albumin as standard. Neutral protease activity levels and free amino acid content were estimated by the method of Moore and Stein (1954). Glutamate dehydrogenase (GDH) was assayed by the method of Lee and Lardy (1965). Ammonia, urea and glutamine were estimated by the methods of Bergmeyer (1965), Natelson (1971) and Colowick and Kaplan (1957), respectively. The data were subjected to statistical analysis as per Bailey (1965).

3. Results and discussion

The data presented in tables 1–3 show the changes in certain parameters of midgut gland, muscle and gill tissue nitrogen metabolic profiles during acute and chronic exposure to a sublethal concentration of phosphamidon.

On acute exposure to subacute concentration of phosphamidon, the total protein content of the midgut gland, muscle and gill tissues was considerably reduced. The sucrose soluble protein content was not significantly, whereas the sucrose insoluble protein content was significantly reduced. The neutral protease activity levels was elevated with a concomitant increment in the free amino acid content. GDH activity levels was also reduced but changes in the levels of ammonia, urea and glutamine were insignificant.

On chronic exposure to a sublethal concentration of phosphamidon, all the tissues showed a significant increment in the total, sucrose soluble and insoluble protein contents. Neutral protease levels were found to be significantly elevated but considerable reduction was observed in the free amino acid content. GDH activity levels was also elevated, whereas ammonia and urea were significantly decreased but glutamine content was significantly increased.

Acute exposure of phosphamidon causes depletion in the total protein content and this decrease might be due to increased proteolytic activity or enhanced entry of various proteins into hemolymph (Sreenivasula Reddy *et al* 1983b). The increased neutral proteolytic activity levels suggestive of the onset of degradable activities in all the tissues of phosphamidon exposed (PE) prawns, due to acute toxic impact of

Table 1. Changes in certain parameters in selected tissues of control and Phosphamidon exposed prawn.

		Exposure time (days)			
		Acute	Chronic		
Tissue	Control	2	10	20	30
Total proteins					
Midgut gland	185·41 ± 14·49	121·18 ± 10·12	164·23 ± 18·44	288·13 ± 21·18	312·62 ± 16·18
Muscle	228·90 ± 20·34	165·24 ± 18·42	301·38 ± 29·44	334·04 ± 31·31	364·32 ± 26·22
Gill	112·15 ± 12·10	84·42 ± 6·75	140·83 ± 10·42	162·18 ± 11·75	170·23 ± 11·45
Soluble proteins					
Midgut gland	120·45 ± 10·31	101·34[a] ± 10·15	189·21 ± 14·18	202·17 ± 15·34	234·44 ± 18·36
Muscle	69·95 ± 7·58	61·14[a] ± 5·84	112·25 ± 12·39	123·18 ± 13·45	141·22 ± 14·84
Gill	73·18 ± 6·05	62·48[a] ± 5·85	94·82 ± 6·71	115·72 ± 7·18	130·42 ± 7·48
Insoluble proteins					
Midgut gland	64·15 ± 6·13	43·41 ± 5·03	91·34 ± 7·89	100·43 ± 10·15	121·52 ± 10·86
Muscle	148·75 ± 13·42	102·34 ± 12·89	202·11 ± 18·44	228·19 ± 15·49	252·15 ± 15·18
Gill	51·11 ± 4·12	30·05 ± 3·12	61·23 ± 5·02	73·43 ± 6·09	80·12 ± 6·81

Each value is mean of 6 individual observations ± indicates SD. Values in parentheses are per cent alterations over control. All values are statistically significant at $P < 0{\cdot}001$ except [a]$P < 0{\cdot}01$.
Values are expressed as mg/g wet wt.

Table 2. Changes in certain parameters in selected tissues of control and phosphamidon exposed prawn.

		Exposure time (days)			
		Acute	Chronic		
Tissue	Control	2	10	20	30
Protease (μ mol of tyrosine formed/mg protein/h)					
Midgut gland	1·19 ± 0·18	1·62 ± 0·20	1·94 ± 0·28	2·09 ± 0·31	2·26 ± 0·29
Muscle	0·85 ± 0·12	1·12 ± 0·25	1·29 ± 0·32	1·34 ± 0·28	1·46 ± 0·29
Gill	0·62 ± 0·11	0·72[a] ± 0·12	0·81 ± 0·13	0·93 ± 0·14	1·05 ± 0·15
Free amino acids (μ mol/g wet wt)					
Midgut gland	94·17 ± 8·42	141·28 ± 12·49	51·44 ± 4·53	38·19 ± 5·13	32·15 ± 4·12
Muscle	185·23 ± 15·45	244·23 ± 23·49	102·14 ± 18·42	98·09 ± 5·44	87·82 ± 6·03
Gill	60·18 ± 4·42	85·75 ± 5·81	51·22 ± 3·88	46·82 ± 3·81	34·12 ± 2·89
GDH (μ mol of formazan formed/mg protein/h)					
Midgut gland	1·43 ± 0·15	1·01 ± 0·12	1·83 ± 0·24	2·03 ± 0·21	2·38 ± 0·23
Muscle	0·78 ± 0·05	0·61 ± 0·08	0·96 ± 0·09	1·05 ± 0·13	1·31 ± 0·14
Gill	1·02 ± 0·12	0·86[b] ± 0·09	1·33 ± 0·12	1·43 ± 0·15	1·51 ± 0·17

Each value is mean of 6 individual observations ± indicates SD. Values in parentheses are per cent alterations over control. All values are statistically significant at $P < 0{\cdot}001$ except [d]Not significant; $P < 0{\cdot}01$.

phosphamidon, with a concomitant reduction in the total protein content. The sucrose insoluble protein content showed a significant reduction in its content suggestive of higher degree of proteolysis at the structural organization of PE prawn tissues viz midgut gland, muscle and gill. The total free amino acid content was increased significantly which might be due to increased proteolysis as indicated by the results tabulated in table 2 or due to decreased utilization in oxidative metabolic

Table 3. Changes in certain parameters in selected tissues of control and phosphamidon exposed prawn.

		Exposure time (days)			
		Acute	Chronic		
Tissues	Control	2	10	20	30
Ammonia					
Midgut gland	10·45 ± 1·21	11·52^{a} ± 1·10	4·18 ± 0·55	3·94 ± 0·41	3·22 ± 0·40
Muscle	5·32 ± 0·84	6·02^{a} ± 0·58	2·84 ± 0·61	2·71 ± 0·50	2·52 ± 0·48
Gill	6·08 ± 0·74	7·05^{b} ± 0·71	4·05 ± 0·32	3·08 ± 0·25	2·91 ± 0·28
Urea					
Midgut gland	1·19 ± 0·23	1·08^{a} ± 0·20	0·61 ± 0·13	0·54 ± 0·11	0·43 ± 0·12
Muscle	1·059 ± 0·152	0·992^{a} ± 0·101	0·589 ± 0·085	0·554 ± 0·081	0·548 ± 0·088
Gill	0·814 ± 0·051	0·790^{a} ± 0·060	0·515 ± 0·055	0·445 ± 0·051	0·401 ± 0·048
Glutamine					
Midgut gland	21·44 ± 20·35	24·15 ± 2·13	58·78 ± 8·15	63·41 ± 7·85	75·08 ± 6·73
Muscle	6·72 ± 0·89	7·02^{a} ± 0·75	16·49 ± 1·42	19·34 ± 1·84	23·18 ± 2·04
Gill	10·08 ± 0·95	11·42^{b} ± 1·13	17·84 ± 1·80	21·80 ± 1·95	29·13 ± 2·05

Each value is mean of 6 individual observations ± indicates SD. Values in parentheses are per cent alterations over control. All values are statistically significant at $P < 0{\cdot}001$ except ^{a}Not significant; $^{b}P < 0{\cdot}02$; $^{c}P < 0{\cdot}05$. Values are expressed as μ mol/g wet wt.

reactions (Srinivasulu Reddy *et al* 1983a; Bhagyalakshmi *et al* 1984). A significant reduction in the activity levels of GDH, which forms an index of amino acid oxidation into Kreb's cycle, the decreased mobilization of amino acid into oxidation can be expected. Similar kind of observations were reported in crabs under sumithion toxicity (Sreenivasula Reddy *et al* 1982). The contents of ammonia, urea and glutamine were not significantly affected, indicative of the probability of least disturbance in the formation and excretion of nitrogenous end products of all the PE prawn tissues in response to acute exposure of phosphamidon.

A significant elevation in the total, sucrose soluble and insoluble protein content suggesting the onset of their increased protein biosynthesis of PE prawn tissues or decreased proteolytic activity. But the protease activity levels was found to be shown a significant increment. The total free amino acid content showed a considerable reduction, which might be due to increased operation of transamination reactions under pesticide stress (Bhagyalakshmi *et al* 1984). GDH activity levels was significantly increased. Inspite of increased oxidative deamination as evidenced by increased activity levels of GDH, ammonia content was shown to be considerably decreased. Most probably the mobilization of ammonia towards the formation of less toxic substances like urea or glutamine may be one of the adaptive mechanisms by which it detoxifies the ammonia present at the cellular environment of PE prawn tissues (Bhagyalakshmi 1980). But interestingly, urea showed a decrement indicative of urea biosynthesis from ammonia was ruled out.

From the present study it can be concluded that even the low concentration of phosphamidon was able to alter the tissue metabolic profiles of PE prawn tissues during acute (2 day) and chronic (10, 20 and 30 days) exposure. All the PE tissues have shown efficient mechanisms of detoxification by shifting its nitrogen metabolic patterns and also adopting compensatory mechanisms like increased biosynthesis of various kinds of proteins and also detoxification of toxic substances like ammonia to relatively nontoxic substances will pave way for the survivability of these prawns in the phosphamidon contaminated environments.

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Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos)

V UMA DEVI, Y PRABHAKARA RAO and D G V PRASADA RAO
Department of Zoology, Andhra University, Waltair 530 003, India

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Abstract. The snail *Morula granulata* (Duclos) survived the starvation stress over a period of 30 days but 50% mortality occurred on the 70th day. The changes in the level and content of different biochemical constituents viz carbohydrates, glycogen, free sugars, protein, total ninhydrin positive substances and lipids were investigated in foot, gonad digestive gland complex and viscera of the animal at intervals of 10 days up to 70 days. All these biochemical constituents (both level and content) were found to decrease with the period of starvation showing their utilisation except total ninhydrin positive substances which exhibited an increase. Of all the 3 tissues, the gonad digestive gland complex was affected most during starvation. 'Lipid-oriented' metabolism was observed in this animal showing maximum utilisation of lipids during starvation. Respiratory studies revealed a decreased pattern of oxygen consumption with increasing period of starvation when calculated per snail or per tissue weight. The recalculated intercept values (a) also showed a decreasing trend when exposed to starvation.

Keywords. Starvation stress; biochemical constituents; tropical intertidal gastropod; lipid oriented metabolism; oxygen consumption

1. Introduction

Lack of food constitutes a stress for the animal and the ability of the animal to withstand this nutritive stress for prolonged periods depends on its ability to utilise the stored nutrients economically. A good quantity of information is available on the effect of starvation stress in molluscs (Martin and Goddard 1966; Giese 1966; Emerson 1967; Emerson and Duerr 1967; Stickle and Duerr 1970). Interestingly, some of the gastropods show polysaccharide-oriented metabolism (Barry and Munday 1959) whereas in other gastropods, it is lipid-oriented (Stickle and Duerr 1970).

Several investigations on the starvation metabolism of gastropod molluscs revealed that, though there is a preferential utilisation of one particular body reserve during the course of starvation typical for a particular species (Emerson 1967; Stickle and Duerr 1970; Ricardo and Brennes 1981), the other products also are being utilised equally or to a lesser extent. Thus work was done on several types of snails where specifications exist among the various types of gastropods.

Although the gastropods were studied extensively, there appears to be little information on the nutrient stores and respiration in relation to starvation of either carnivorous prosobranch snails or opisthobranch snails. Therefore, in the present investigation, an attempt has been made to study the level and content of organic constituents, and respiration in a carnivorous snail *Morula granulata* (Duclos) during starvation.

2. Materials and methods

Animals of uniform size (dry weight of soft parts ranged from 80–150 mg) were collected from the rocks of Palm beach of Visakhapatnam on east coast of India. They were equilibrated in the laboratory in an aquarium containing sea water (32‰) at 25° ± 0·5°C for 24 h.

Preliminary investigations were carried out to study the mortality rate of animals exposed to starvation stress. A batch of 100 animals were taken in an aquarium filled with Whatman 42 filtered sea water. The filtered sea water was aerated continuously and the water was changed daily. Observations were made for intervals of 10 days up to 70 days where 50% mortality occurred.

2.1 *Sampling technique*

For biochemical studies, a group of 300 animals were taken in an aquarium and 20 animals from this group were sacrificed which served as initial (0 days). The rest of the animals were subjected to starvation stress as explained above. Sampling intervals were fixed for every 10 days up to 70 days depending on the above observations on mortality rate. At each interval, 20 animals were sacrificed for biochemical analysis.

Immediately after sacrificing the animals, the different body components viz foot, gonad digestive gland complex (GDG complex) and viscera were excised and pooled up separately. As the gonad and digestive gland are inseparable they were considered as GDG complex hereafter. Wet weights of the pooled body components were taken before they were dried in an oven at 90°C for 48 h to a constant weight and dry weights of all the samples were also taken. The dried samples were powdered and were kept in glass vials stored in a desiccator. The biochemical composition of each component in *M. granulata* was estimated separately at various stages of starvation.

2.2 *Biochemical analysis*

Anthrone method was followed for the estimation of total carbohydrates and glycogen (Carrol *et al* 1956). The difference between the amount of total carbohydrates and glycogen was taken as free sugars. Protein content was estimated by the method of Lowry *et al* (1951). Total ninhydrin positive substances (TNPS) were estimated using the method of Moore and Stein (1954). Total lipids were determined by applying the chloroform: methanol (2:1) extraction procedure described by Folch *et al* (1957).

2.3 *Biochemical level and content*

The different biochemical constituents are presented as mg/g dry weight of the tissue (biochemical level). The biochemical content (total present per component of a 100 g standard animal) was estimated by the method of Stickle (1975). The body component indices of *M. granulata* subjected to starvation stress have been reported earlier by Uma Devi *et al* (1985).

2.4 *Respiration*

For respiratory studies, 10 animals of equal size (as described earlier) were selected and labelled serially from 1–10. Their initial oxygen consumption was measured and then they were subjected to lack of food by keeping them in Whatman 42 filtered sea water (salinity 32%, pH 8). The animals were kept in a glass trough at a temperature of 25° ± 0·5°C with continuous aeration. Oxygen consumption was studied for all the 10 animals individually at 10 days intervals (10, 20, 30, 40, 50, 60 and 70) over a period of 70 days under starvation at the same temperature (25°C). Respiratory measurements were made according to the method used by Prasada Rao and Uma Devi (1981). After measuring the oxygen consumption, they were transferred back to the experimental troughs.

2.5 *Statistical evaluation*

All the data are presented as mean ± standard deviation. One way analysis of variance (ANOVA) (Snedecor and Cochran 1967) was used to determine the significance of variation in biochemical level and content during starvation. Duncan's Multiple Range test (Snedecor and Cochran 1967) was used to study the above data further. Respiratory rates were also studied by using the above test.

3. **Results**

Mortality data (cumulative) with reference to starvation are given in table 1. 50% mortality was recorded after 70 days of starvation. Based on this observation, the present investigation has been planned.

3.1 *Biochemical level*

The changes in the level of biochemical constituents of foot, GDG complex and viscera are presented in figures 1–3. From ANOVA, it is clear that there are significant variations ($P < 0{\cdot}05$) in all the biochemical constituents of different body components. Carbohydrates decreased significantly ($P < 0{\cdot}05$) from 30, 40 and 50

Table 1. Effect of starvation on the respiration of *Morula granulata* (see text for details of oxygen consumption and log *a* values).

No. of Days	Mortality (%)	Oxygen consumption μl $O_2 \cdot h^{-1}$ ± SD	Decrease (%)	Oxygen consumption μl $O_2\ g^{-1} \cdot h^{-1}$ ± SD	Decrease (%)	log *a*
0	0	58·93 ± 8·18	—	0·3982 ± 0·0553	—	0·8193
10	0	49·10 ± 10·07	16·68	0·3672 ± 0·0753	7·79	0·7594
20	0	44·19 ± 9·88	25·01	0·3710 ± 0·0830	6·83	0·7356
30	0	39·57 ± 9·04	32·85	0·3365 ± 0·0769	15·50	0·6901
40	10	34·15 ± 7·73*	42·05	0·3012 ± 0·0682*	24·36	0·6331
50	20	27·90 ± 5·50*	52·66	0·2527 ± 0·0498*	36·54	0·5504
60	35	18·92 ± 3·37*	67·89	0·1775 ± 0·0316*	55·42	0·3883
70	50	18·50 ± 3·64*	68·81	0·1823 ± 0·0359*	54·22	0·3880

*$P < 0{\cdot}05$

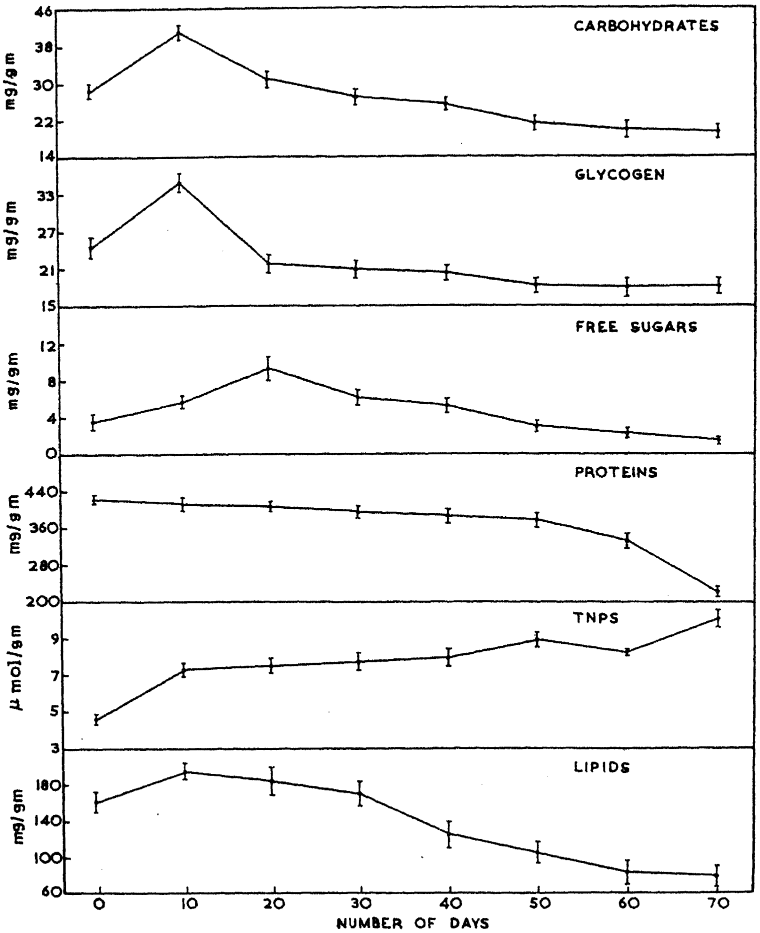

Figure 1. Effect of starvation on the biochemical composition (level) of foot in *M. granulata* (vertical bars represent the standard deviation).

days in foot, GDG complex and viscera, respectively. Significant decrease ($P < 0{\cdot}05$) in glycogen levels of foot was observed from the same period as for carbohydrates but in GDG complex, the decrease in glycogen level occurred at 20 days and it continued up to 70 days. In viscera, the same trend was noticed as in foot. The free sugars of foot and viscera decreased ($P < 0{\cdot}05$) from 50 days onwards. The protein levels of GDG complex and viscera showed gradual decrease ($P < 0{\cdot}05$) immediately after 10 days. In foot, significant decrease of protein levels was observed from 40 days. The TNPS levels were found to increase gradually in foot, GDG complex and viscera and this increase was significant from 40 days. In all the body components studied, the lipid levels decreased significantly ($P < 0{\cdot}05$) from 50 days onwards.

3.2 *Biochemical content*

ANOVA of different body biochemical contents reveals significant variations ($P < 0{\cdot}05$) in their composition during different intervals of starvation. However, it is

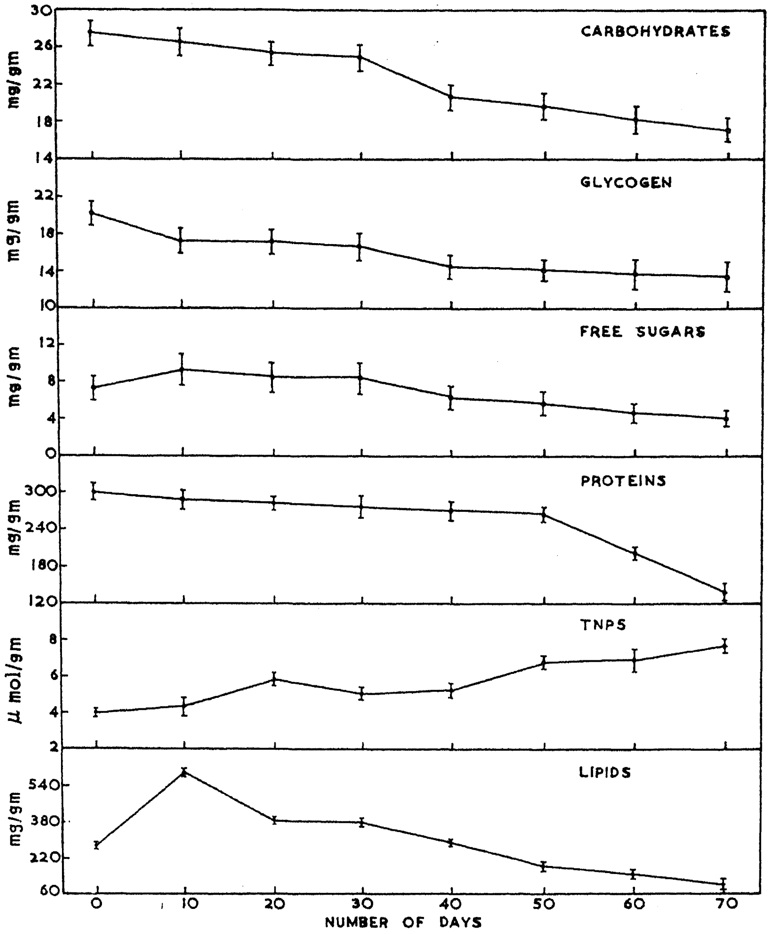

Figure 2. Effect of starvation on the biochemical composition (level) of GDG complex in *M. granulata* (vertical bars represent the standard deviation).

evident from Duncan's analysis that carbohydrates, glycogen, free sugars, proteins and lipids decreased with increasing period of starvation in foot, GDG complex and viscera as was observed earlier for biochemical levels. The significant increase ($P<0{\cdot}05$) in the content of TNPS is similar to that of level in all the body components during starvation.

It is clear from the results that the lipid quantities exhibited significant decrease ($P<0{\cdot}05$) in all the body components of *Morula granulata*. Therefore, it can be concluded that this snail belongs to the category of molluscs showing lipid-oriented metabolism when exposed to starvation stress.

3.3 *Respiration*

The details of oxygen consumption of *M. granulata* during different intervals of starvation are presented in table 1. It is clear from the percentage calculation that the decrease in oxygen consumption, when considered on per animal basis, was gradual

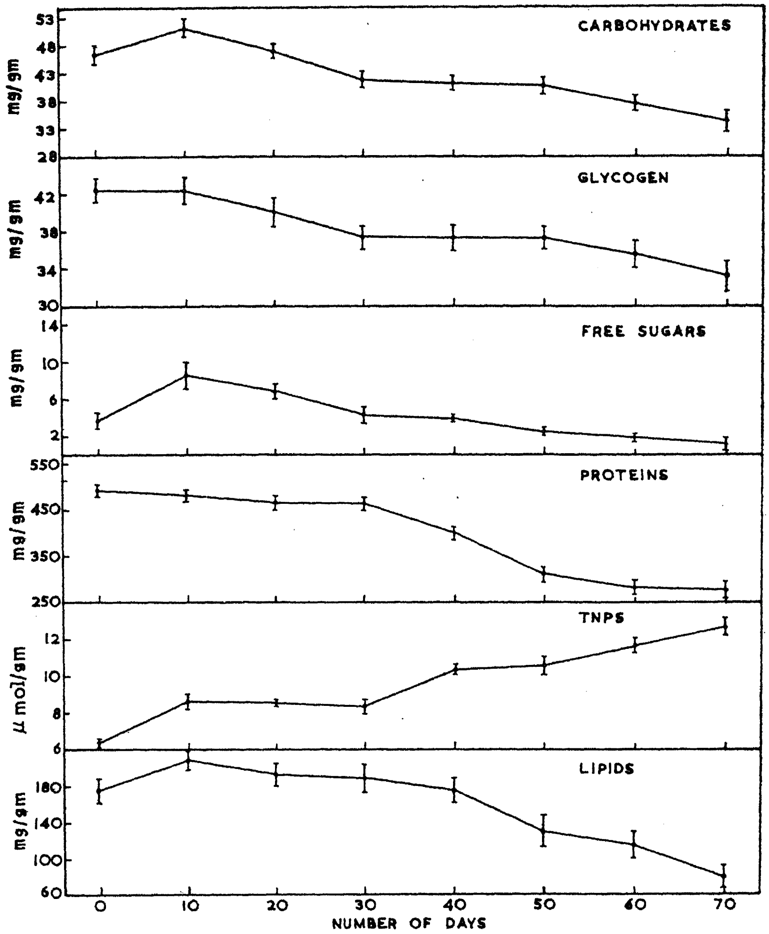

Figure 3. Effect of starvation on the biochemical composition (level) of viscera in *M. granulata* (vertical bars represent the standard deviation).

with the period of starvation. From Duncan's multiple range test, it is found that the decrease is significantly different ($P < 0.05$) from 40 days of starvation. The oxygen consumption values when calculated basing on tissue weight (this was estimated from percentage decrease in dry tissue weights during starvation), also showed a decreasing trend. But this percentage decrease was less when compared to per animal basis (table 1). The log a values of exponential equation $Y = aW^b$ are calculated by using the 'b' value of 0·4382 reported earlier for this animal (Prasada Rao and Uma Devi 1981). These log a values are also found to decrease with increasing period of starvation.

4. Discussion

Mortality rate of *M. granulata* is very slow (table 1) when subjected to prolonged starvation of 70 days. This clearly indicates that this animal has good capacity to withstand starvation stress. This is in agreement with previous work of Stickle and Duerr (1970) on *Thais lamellosa.*

The organic constituents of soft parts of *M. granulata* during starvation stress show trends of diminution with increase in the duration of the stress conditions. The decrease of these materials is somewhat slow during the earlier period of starvation, after which the decline is at a rapid rate (figures 1–3). Of all the principal biochemical constituents, the highest decrease was found for lipids (73·85% for content and 63·35% for level) in GDG complex (figure 2). However, this decrease was comparatively less in foot (70·97% for content and 52·03% for level) (figure 1) and viscera (61·71% for content and 54·62% for level) (figure 3). A remarkable decrease was observed for proteins but not as distinct as lipids (67·11% for content and 53·89% for level in GDG complex; 68·50% for content and 47·44% for level in foot). In viscera, proteins exhibited a decrease of 52·77% for content and 44·02% for level. The decrease in the percentage of total carbohydrates was: 58·70 for content and 31·86 for level in foot, 55·35 for content and 37·52 for level in GDG complex, 37·32 for content and 25·77 for level in viscera. However, glycogen and free sugars followed the same trend as explained above in different tissues (figures 1–3). Thus it is interesting to observe that during starvation, the percentage decrease of different nutrient classes is more when considered on basis of content than on the level. It may also be said that the effect of starvation is high on the GDG complex when compared to the other tissues.

A close examination of the data reveals that it is the lipid that is much reduced by starvation stress. Next to lipids, protein utilisation is more in *M. granulata.* In this study, total carbohydrates and glycogen occupy the last place in their role as a source of food when compared to other body constituents during starvation. The results of this investigation strongly suggest that the metabolism of the carnivorous prosobranch *M. granulata* is 'lipid-oriented'. This is in agreement with the previous work on a carnivorous prosobranch *Thais lamellosa* (Stickle and Duerr 1970) and on the intertidal prosobranch *Littorina planaxis* (Emerson and Duerr 1967).

Pulmonates appear to possess a polysaccharide-oriented metabolism (Emerson 1967) while prosobranchs have a lipid-oriented metabolism (Emerson and Duerr 1967). Pulmonate and prosobranch snails differ from each other not only morphologically but also in an equally fundamental metabolic way (Stickle and Duerr 1970).

Starvation results in an elevation of free amino acid (TNPS) level of the soft parts of *M. granulata.* This increase in free amino acids, as a result of proteolysis, would benefit the animal in two ways, one way that might be utilised as precursor for gluconeogenesis under stress conditions to meet the energy demands; secondly, since starvation is a stress condition imposed on the animal, the inherent ability of the molluscs to withstand the adverse condition in their nourishment depends on the capacity to regulate the ionic and osmotic concentrations of both blood and cells (Schoffeniels and Gilles 1972), so that the proteolysis should help to maintain the osmoregulatory balance. Free amino acids could also be utilized for the purpose of energy needs.

There is a continuous reduction in the total metabolism with progressive starvation up to 70 days. A decreased trend in the oxygen consumption in relation to starvation was observed by Berg *et al* (1958) in freshwater limpet *Ancylus fluviatilis,* Duerr (1965) in *Lymnaea palustris,* Lumbye and Lumbye (1965) in *Littorina planaxis.*

Although, a similar decrease in oxygen consumption during long term starvation has been reported in *Thais lapillus* (Stickle and Bayne 1982), no change was observed in the recalculated intercept (*a*). However, in the present investigation on *M. granulata*, the recalculated intercept values were found to decrease with increasing period of starvation which corroborates with the results of Bayne and Scullard (1978) on *T. lapillus*. Stickle and Duerr (1970) and Stickle (1971) have observed constant or increased rates of weight specific oxygen consumption in *T. lamellosa* during starvation. However, *M. granulata* exhibited a decreased pattern of weight specific oxygen consumption when exposed to starvation stress. It is possible that the observed decrease in oxygen consumption of *M. granulata* may be an adaptation enabling the snail to conserve the food stored.

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Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India

P S SASIKUMAR, PREMA SURYANARAYANAN*, CHERIYAN THOMAS, K KALYANARAMAN** and R S PRASAD
Department of Zoology, *Department of Geology and **Department of Statistics, University of Kerala, Kariavattom, Trivandrum 695 581, India

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Abstract. A year round study was conducted in Trivandrum city during the period of March 1984 to February 1985 to elucidate the relation between certain physico-chemical factors and seasonal density of *Mansonia* larvae (Culicidae: Diptera). *Mansonia uniformis* was found to be the predominant species during this period, whereas *Mansonia annulifera* was less than 10% of the total *Mansonia* collected. The period of high prevalence was found to be January–March. Out of the 6 physico-chemical factors studied Na^+, K^+, and pH showed statistically significant correlation with the larval density, while Ca^{++}, Mg^{++} and rainfall showed no significant relation.

Keywords. *Mansonia*; larval density; physico-chemical factors.

1. Introduction

Mansonia mosquitoes are known to be the principal vectors of Brugian filariasis in Kerala. They are found to breed in water rich in decaying organic matters (Burton 1959). The existing information shows that 3 species of *Mansonia* viz *Ma. annulifera*, *Ma. uniformis* and *Ma. indiana* are prevalent in Kerala. The former two have been incriminated as vectors of Brugian filariasis and are present in Trivandrum City (Jaswant Singh *et al* 1956; Nair and Roy 1958; Nair 1962; Chandrasekharan *et al* 1976). The attachment preference of *M. annulifera* and *M. uniformis* larvae to various aquatic plants in Kerala was studied in detail by Burton (1959). Despite the importance of *Mansonia* in the transmission of the disease, relatively little is known about the population dynamics of these mosquitoes in relation to physico-chemical factors.

The present study relates to the seasonal variations in larval population of *Mansonia* mosquitoes in Trivandrum City, and its relationship, if any, to certain physico-chemical factors of the larval habitats.

2. Material and methods

2.1 *Study area*

The present study was carried out in a low lying area (Kannanmoola) within Trivandrum City limits. The canal which runs through the study area is stocked with aquatic plants like *Eichhornia speciosa* affording continuous breeding of *Mansonia* mosquitoes. In the study area the canal is about 3 metres deep with running water in the centre and the vegetation stocked on both sides. This canal empties into a lake approximately 3 km away from the study area. Larval collections were made from different parts of the canal and nearby uncultivated paddy field with growth of aquatic vegetation such as *E. speciosa*.

2.2 *Larval collection*

Larval collections were made during the period of March 1984 to February 1985. Since *Mansonia* larvae are found attached to the roots of aquatic plants (in the present case *E. speciosa*), the plants were removed and shaken vigorously in a plastic basin containing water. The detached larvae were picked up using glass droppers from the silt and counted (Gillet 1971). Collections were made twice a week between 8·30 and 11·30 am. About 150 plants were examined at each collection. Larval density was calculated by computing the number of larvae/plant. As the larvae had to be used for further studies in the laboratory no attempt was made to identify the species of *Mansonia* larvae for fear of injuring them and minimum disturbances to the field collected larvae was preferred. The larvae were held in the laboratory until emergence and the proportion and pattern of emergence were studied.

2.3 *Chemical analyses of water samples*

Water samples for chemical analyses were procured from the same area of larval collection. After determining the pH, the samples were assayed for Na^+, K^+, Ca^{++} and Mg^{++}. Flame photometric method was employed using MK-1/MK-11/Type 125 Systronic for the quantitative analysis of Na^+ and K^+ whereas Complexometric titration was employed using EDTA as primary standard for Ca^{++} and Mg^{++} (American Public Health Association 1960). Water samples for the study were collected twice a month (during the first and last week of each month).

2.4 *Statistical analyses*

Statistical analyses for possible correlation between pH, ionic concentration of Na^+, K^+, Ca^{++}, Mg^{++} and fluctuations of larval density were studied by using Simple Correlation, Partial Correlation and Student's '*t*' test. Though larval collections were made twice a week, the data used for statistical analyses were only those of the day on which water collections were made.

3. Results

From 2826 *Eichhornia* plants screened during the period of study (March 1984 to February 1985), a total of 11495 *Mansonia* larvae were recovered. The major peak of larval density was during January to March, whence there was a sharp decline followed by a slight increase in density during the period August–October. However, the increase in density during August–October was not as high as that of January–March. The maximum larval density (9·45 larvae/plant) was found in March and the minimum (1·11 larvae/plant) in October (figure 1, table 1).

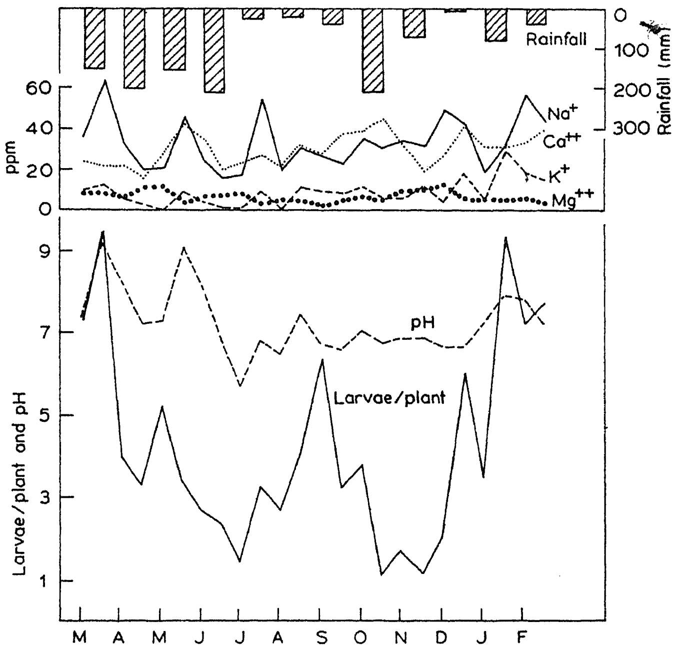

Figure 1. Fluctuations of larval population, pH, Na^+, K^+, Ca^{++}, Mg^{++} and rainfall during 1984 March to 1985 February.

3.1 *Chemical analyses of water*

The results of the chemical analyses are given in figure 1 and table 1. The pH of the water samples ranged from 5·76 to 9·2. Larval density and pH showed a positive correlation which was significant at 5% level. In other words the density of *Mansonia* larvae reached its peak when pH was >7 and it was lowest when pH was <7.

As it is evident from figure 1 wide fluctuations were observed in the concentration of Na^+ and Ca^{++} but not in the concentration of Mg^{++} and K^+. Statistical analyses showed that there is a positive correlation between the larval density and the concentration of K^+ and Na^+. The influence indicate a rise in larval density corresponding to a rise in concentration of these ions. This was found more applicable with regard to the concentration of K^+ (significant at 1% level), which showed a clear increase during the months of January, February and March, the 3 months of highest larval density. From table 1 and figure 1 it is evident that the concentration of K^+ showed an increase toward the end of January (29 ppm). The increase was sustained in February (average 17 ppm) and March (average 11·5 ppm). Na^+ does not exhibit such a clear cut correlation though statistically significant at 5% level. Though not statistically significant the coefficient of correlation with regard to Mg^{++} and larval density gave negative value (table 2). Statistical analyses did not show any significant correlation between monthly larval density and monthly total rain fall.

Na^+ and K^+ appeared to have a mutual influence quantitatively as it is evident from the correlation matrix (table 2). When the concentration of Na^+ increases there

Table 1. Physico-chemical factors of *Mansonia* breeding water and larval density.

Month	Larval density/plant	Na^+ ppm	K^+ ppm	Ca^{++} ppm	Mg^{++} ppm	pH	Rainfall (mm)
1984 March	7·29	36	10	24	9	7·42	151
	9·45	64	13	22	9	9·20	
April	3·96	34	6	22	7	8·20	197
	3·31	20	3	16	12	7·23	
May	5·23	21	0	28	12	7·32	153·7
	3·41	47	10	44	5	9·10	
June	2·70	25	5	36	7·3	8·11	205·5
	2·35	16	2	20	7·5	6·75	
July	1·47	18	2	24	9	5·76	26
	3·25	15	10	28	4	6·85	
Aug.	2·71	21	2	22	6	6·50	21
	4·12	32	12	32	5	7·50	
Sept.	6·37	28	10	28	3	6·81	40·2
	3·32	24	9	38	5	6·67	
Oct.	3·79	36	12	40	7·5	7·09	205·1
	1·11	32	6·8	46	6	6·80	
Nov.	1·68	35	7	34	10	6·92	71·8
	1·55	32	12	20	11	—	
Dec.	2·07	50	5	28	13·3	6·72	2·7
	6·07	43	18	42	6	—	
1985 Jan.	3·54	19	6	32	6	7·28	81·7
	9·38	33	29	32	5	7·93	
Feb.	7·28	57	19	34	6	7·86	40·2
	7·75	44	15	40	3·8	7·27	

Table 2. Results of statistical analyses.

	L	Na^+	K^+	Ca^{++}	Mg^{++}	pH
L	1	0·446^{a} (2·337)	0·673^{b} (4·268)	0·038	−0·297	0·497^{a} (2·68)
Na^+		1	0·522^{a} (2·869)	0·238	−0·109	0·484^{a} (2·590)
K^+			1	0·358	−0·438^{b} (4·73)	0·329
Ca^{++}				1	−0·480^{b} (4·80)	0·105
Mg^{++}					1	−0·098
pH						1

Number in parentheses indicates '*t*' value; ^{a}Significant at 5% level; ^{b}Significant at 1% level. *Mansonia* larvae per *E. speciosa*.

is a corresponding increase of K^+ also. A positive correlation of Na^+ and pH was also evident. When the concentration of Na^+ increases a corresponding increase of pH also was observed. A negative correlation seems to exist between K^+/Mg^{++} and Ca^{++}/Mg^{++}. The *t*-values relating to these correlations showed a statistical significance at 1% level. When the concentration of one ion increases the concentration of the other decreases (figure 1).

4. Discussion

The results of the adult collection from the field (Daniel *et al* 1986) as well as emergence of adults in the laboratory from the field collected larvae revealed *Ma. uniformis* to be the predominating species constituting 90% of the total *Mansonia* collected, the rest being *Ma. annulifera.* This clearly indicate that *Ma. annulifera* is an unimportant member in the *Mansonia* population in the study area. Though larval identification was not carried out in the present work these observations indicate that the species involved in larval analysis was mainly *Ma. uniformis. Pistia stratiotes* was found to be the favourable host plant for *Ma. annulifera.* But the replacement of *P. stratiotes* by *Salvinia auriculata* in many places of Kerala dropped the incidence of *Ma. annulifera* considerably (Joseph *et al* 1963; Chandrasekharan *et al* 1976). Our earlier studies have indicated that the reduction in *Ma. annulifera* populations has some correlation with the disappearance of *P. stratiotes* for the study area. Collections made in the study area during 1983 showed that the population of *P. stratiotes* was more and so was the population of *Ma. annulifera.*

The existing literature on the biology of *Mansonia* mosquitoes shows that the type of correlation between the physico-chemical factors and seasonal larval density of *Mansonia* brought out in the present study has not been elicited earlier.

The present study revealed that out of the 6 physico-chemical factors analysed (Na^+, K^+, Ca^{++}, Mg^{++}, pH and rainfall) 3 (Na^+, K^+ and pH) had significant influence on the density of *Mansonia* larvae. However the studies conducted by Khamala (1971) showed that the physical and chemical nature of water (sedimentary solids, suspended solids, dissolved organic matter, total nitrogen and pH) did not appear to be significant for *Ma. africana* and *Ma. uniformis.*

Earlier workers have presented contradictory views about the seasonal prevalence of *Mansonia* mosquitoes. Studies carried out at Ernakulam showed that the densities of both *Ma. annulifera* and *Ma. uniformis* were higher during post monsoon months (Pal *et al* 1960). However, according to Chandrasekharan *et al* (1976) *Ma. annulifera* is a premonsoon species (January–June) whereas *Ma. uniformis* is a postmonsoon species (July–November). In the present study the high prevalence of *Mansonia* larvae was noticed from January–March and low in July, October and November. The adult collections made in the study area also showed a similar pattern of prevalence (Daniel *et al* 1986).

Iyengar (1938) suggested that breeding of *Mansonioids* is reduced following heavy rainfall due to dilution of organic matter and breeding is dense if there is low or no rainfall. Similar observations were made in *Culex pipiens fatigans* also (Sarkar *et al* 1978). But the present study does not support such a contention.

It appears from the present study that, like the larvae of *Aedes vittatus, Ae. albopictus, Ae. aegypti* (Sehgal and Pillai 1970) and *Cx. p. fatigans* (De-Alwis and Munasinghe 1971; Kaul *et al* 1977; Sinha 1976; Sarkar *et al* 1978), *Mansonia* larvae are also alkilinophilic.

The tolerance of mosquito larvae to various elements differ from species to species. Hangstrum and Gunstream (1971) examined the concentration of inorganic ions in water samples from a large number of sources in relation to 8 species of mosquito larvae. They separated these species of mosquitoes into two groups, 'Species limited' and 'Species wide spread' in their distribution. The results showed that the widely distributed species of mosquitoes must be less selective and have a broader range of tolerance for inorganic ions than species of limited distribution. Salinity of the natural breeding water analysed in February (the period of peak larval density) showed to be 0·076 g of NaCl/litre (WHO 1975). A laboratory study using artificial sea water showed that the larvae of both species of *Mansonia* were able to tolerate a dilution of 6 × and above (unpublished observation). Sodium chloride concentration of 6 × diluted artificial sea water would be about 5·36 g of NaCl/litre. It thus appears that *Mansonia* larvae can tolerate a wide range of salinity. How far the larvae are exposed to such a wide range of salinity variation in their natural breeding areas is yet to be elucidated.

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Adaptedness of five closely related strains of *Drosophila* to media containing molasses or fructose

A USHAKUMARI and H A RANGANATH
Department of Post-Graduate Studies and Research in Zoology, Manasagangotri, University of Mysore, Mysore 570 006, India

MS received 9 February 1985; revised 18 April 1986

Abstract. Relative performance of morphologically indistinguishable and phylogenetically closely related forms of *Drosophila* was assessed in two different media. The flies performed better in media with molasses than in media with fructose. There were significant differences in the ability of these strains to exploit a similar sugar resource suggesting differences in the adaptedness of these forms. The implications of these findings are discussed.

Keywords. *Drosophila*; sugars; adaptedness.

1. Introduction

Drosophila species utilize a variety of sugar sources (Hassett 1948; Taylor and Condra 1983). The extent to which populations are differentiated in relation to such resources is not known. We herein report the 'adaptedness' of the members of the orbital sheen complex of the *nasuta* subgroup of the *immigrans* species group of *Drosophila* in two different media containing different types of sugars.

The orbital sheen complex is an assemblage of morphologically similar forms of *Drosophila*. The members of this complex include *D. sulfurigaster sulfurigaster*, *D. s. albostrigata*, *D. s. bilimbata*, *D. s. neonasuta* and *D. pulaua* (Wilson *et al* 1969; Nirmala and Krishnamurthy 1974; Ranganath and Krishnamurthy 1976). The extent of genetic differentiation between them in terms of mating behaviour (Spieth 1969), enzyme variation (Ramesh and Rajasekarasetty 1980), fixed inversion differences (Rajasekarasetty *et al* 1980) and heterochromatin content (Ranganath and Ushakumari 1984) have been recorded. To further these investigations, the present project was undertaken to understand the adaptive differences if any, under laboratory conditions among these phylogenetically closely placed and morphologically identical forms of *Drosophila*.

2. Materials and methods

The strains used were *D. s. sulfurigaster* (P–11, Port Moresby, Papua, New Guinea), *D. s. albostrigata* (S–11, Sandakan, Sabah, Malaysia), *D. s. bilimbata* (HNL–111, Hawaii), *D. s. neonasuta* (209·2 Mysore, India) and *D. pulaua* (S–18, Sandakan, Sabah, Malaysia).

Two types of media were used, viz (i) wheat cream agar medium with molasses and (ii) wheat cream agar medium with fructose. Flies were maintained in these media for 12 weeks. Four replicates were kept for each strain in each media. The populations were cultured at 22°C following the serial transfer technique of Ayala

(1965). Adult flies were introduced into quarter pint milk bottles containing the appropriate medium. Once in 7 days they were etherised, counted and transferred to fresh media bottles. When flies began to emerge in the bottles, the newly emerged flies were etherised, counted and added to the bottle with the adult flies. The bottle was discarded after 4 weeks. The adult ovipositing flies were thus always in a single bottle while other bottles contained different preadult stages of the flies. Four different facets of adaptedness were estimated. They are population size, productivity, flies per bottle and mortality.

3. Results and discussion

Species closely related phylogenetically and phenotypically similar are excellent materials to study the extent of differences if any, in their adaptation to particular environments. Populations of such species can be used in the laboratory as biological models to study the dynamics of adaptive differences.

Populations of organisms must live and reproduce, in a given situation, in order to be designated as adapted. 'Adaptedness' refers to the ability of the carriers of a genotype or a group of genotypes to survive and reproduce in a given environment (Dobzhansky 1968). It is a cumulative estimate of population size, productivity, flies per bottle and mortality. Productivity is the extent of its reproductive potential, measured in terms of new born flies every week. Population size is measured in terms of average population size it maintains during the experimental period. Values for flies per bottle and mortality reflect the average number of flies that survived in each bottle and the number of individuals dying every week, respectively, during the period of assessment.

The relative performance of the members of the orbital sheen complex of *Drosophila* in two different media is presented in tables 1 and 2. The mean values for

Table 1. Mean values (for 4 replicates) along with standard errors for population size, productivity, mortality and flies per bottle in the media with molasses for 5 different strains of *Drosophila*.

Species Parameters	*D. s. sulfurigaster* (P–11)	*D. s. albostrigata* (S–11)	*D. s. bilimbata* (HNL–111)	*D. s. neonasuta* (209·2)	*D. pulaua* (S–18)
Population size	126·44 ± 16·07	132·68 ± 19·09	114·86 ± 3·01	243·29 ± 11·37	159·93 ± 16·24
Productivity	82·58 ± 12·15	106·95 ± 13·52	71·61 ± 4·15	146·38 ± 6·49	120·27 ± 11·55
Mortality	64·70 ± 9·03	86·98 ± 12·65	53·87 ± 2·35	115·35 ± 5·10	102·90 ± 13·06
Flies per bottle	36·78 ± 4·68	38·55 ± 5·59	33·39 ± 0·87	70·75 ± 3·33	46·52 ± 4·75

Table 2. Mean values (for 4 replicates) along with standard errors for population size, productivity, mortality and flies per bottle in the media with fructose for 5 different strains of *Drosophila*.

Species Parameters	*D. s. sulfurigaster* (P–11)	*D. s. albostrigata* (S–11)	*D. s. bilimbata* (HNL–111)	*D. s. neonasuta* (209·2)	*D. pulaua* (S–18)
Population size	7·27 ± 1·26	32·01 ± 6·54	21·98 ± 1·82	69·10 ± 7·36	49·25 ± 2·54
Productivity	0·28 ± 0·28	23·44 ± 6·78	8·74 ± 1·93	26·13 ± 4·03	26·23 ± 2·96
Mortality	2·88 ± 0·24	22·77 ± 5·42	9·33 ± 1·00	18·68 ± 4·46	19·96 ± 2·69
Flies per bottle	2·75 ± 0·54	11·35 ± 2·66	7·16 ± 0·50	21·26 ± 2·26	15·16 ± 0·78

each one of the parameters assessed are given in these tables. To understand the extent of statistical significance between the mean values of the 'population size', the test for analysis of variance was carried out. The test revealed the occurrence of statistically significant differences between the mean values of the 'population size' (in the media with molasses $F = 7{\cdot}53$; $df = 30, 9$; $P < 0{\cdot}01$; in the media with fructose $F = 28{\cdot}38$; $df = 30, 9$; $P < 0{\cdot}01$). Thus, the differential ability of the members of the orbital sheen complex to utilize the media with molasses and the media with fructose is striking. The populations which maintain a larger population size may be said to be performing better from the biological point of view than the one having small population size. This provides means for comparing the overall biological performance of one species with another where both are maintained under similar and/or defined different environmental conditions. In the present experiment, *D. s. neonasuta* has exploited both the media better than the others and it tops the sequence. The ranking for these strains in the media with molasses is: *D. s. neonasuta* > *D. pulaua* > *D. s. albostrigata* > *D. s. sulfurigaster* > *D. s. bilimbata*; and in the media with fructose is *D. s. neonasuta* > *D. pulaua* > *D. s. albostrigata* > *D. s. bilimbata* > *D. s. sulfurigaster*. Similarly Ramachandra and Ranganath (1984) have shown the existence of 'subtle' differences between *D. melanogaster*, *D. ananassae*, *D. n. nasuta* and *D. n. albomicana* in their preference to the media with different types of sugars.

Further, all the strains under investigation have achieved a better expression in the media with molasses than in the media with fructose. Interestingly, *D. s. sulfurigaster* failed to breed in the media with fructose and it virtually reached a point of extinction during the experimental period.

4. Conclusion

The response of the strains measured in terms of 4 different aspects of adaptedness reveal (i) the media with molasses are more suitable for survival and reproduction; (ii) these morphologically indistinguishable forms have demonstrated significant differences in their response to a common type of media. This suggests the possibility of a certain degree of differentiation among these closely related species.

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Seasonal effect on certain developmental stages of *Tricholyga bombycis* Beck. (Diptera: Tachinidae)

PRADIP KUMAR and M S JOLLY
Central Sericultural Research and Training Institute, Mysore 570 008, India

MS received 18 November 1985; revised 9 April 1986

Abstract. Studies conducted for a period of one year (from April 1982 to March 1983) revealed that temperature and relative humidity play a significant role on incubation period, pupal period, emergence percentage, adult longevity and fecundity of uzi fly, *Tricholyga bombycis*. Correlation between temperature, humidity and certain developmental stages is also established.

Keywords. Seasonal effect; developmental stages; *Tricholyga bombycis*.

1. Introduction

Heavy infestation of uzi fly, *Tricholyga bombycis*, is recorded in the intensive sericultural regions of Karnataka and its spread has also been noticed in Andhra Pradesh and Tamil Nadu (Jolly 1981). Occurrence of this parasitic fly is also reported in many other countries of eastern Asia (Ghosh 1949). Although it is mainly a pest of mulberry silkworm, infestation of muga and eri worm by *T. bombycis* has been reported (Mukherjee 1919; Choudhury 1981).

The extent of damage recorded in Karnataka is sometimes upto 40% (Jolly and Kumar 1985). Every year considerable loss of silkworm crops due to uzi fly infestation is reported in West Bengal (Krishnaswami *et al* 1964).

A review of literature reveals that no studies have been made to find out the seasonal effect on the developmental stages of *T. bombycis* in Karnataka one of the most prosperous sericultural tracts of India.

The present investigation was undertaken with a view to understand the influence of temperature and humidity on certain developmental stages of uzi fly during different seasons which may enable us to evolve suitable control measures.

2. Materials and methods

T. bombycis maggots were collected from Ramanagaram area and cultured in the laboratory in netted cages (45 × 45 × 45 cm). The flies were reared on 10% glucose (Glaxo) solution on sterilized cotton wool (Sriharan *et al* 1980).

Silkworms (bivoltine races) were reared in a room (540W × 340L × 315H cm) having an ante-room. All the doors and windows of the room were fixed with wire mesh to ensure that no natural infestation takes place prior to the experimentation. Silkworms were reared as per the recommendations of CSRTI (Krishnaswami 1978). Fifth stadium silkworms from this population were used in this experiment.

Daily temperature and humidity was recorded at 6 h interval in the laboratory. Hatching percentage was obtained based on the number of scars and number of eggs laid. On recovery the maggots were allowed to pupate in loose sterilized soil kept in petridishes (10 cm diameter) till emergence. Silkworms were provided daily to mated female flies for egg laying. Four replications each of 5 pairs were maintained for studies on pupal period, emergence percentage, incubation period, hatching percentage, fecundity and longevity of female flies. Data were recorded once in a month for a period of one year starting from April 1982. Time taken for 50% of the flies to emerge from the day of pupation was considered as pupal period.

The effects of seasonal variations on the developmental stages recorded were statistically analysed (Fisher and Yates 1982) and the relationships existing amongst the various characters were obtained by the correlation coefficient.

3. Results and discussion

3.1 *Incubation period*

The average incubation period was found to be 42 h. The maximum period of 60 h was recorded during June and December and minimum of 30 h during March, April and May (table 1). The incubation period in many species of tachinids is determined as 2–3 days (Clausen 1940).

Statistical correlation of the data indicate negative and highly significant correlation with temperature and positive and non-significant correlation with humidity (table 3). The parameters like hatching percentage and fecundity were positively and non-significantly correlated whereas longevity was positively and highly significantly correlated with incubation period (table 2).

3.2 *Hatching percentage*

An average of 86·50% hatching was recorded. The hatching percentage was maximum (90·78) during August closely followed by 89·96, 89·19 and 89·05 during September, November and July, respectively. The minimum hatching percentage (77·01) was recorded during February (table 1).

The hatching percentage was negatively and non-significantly correlated with temperature and positively and non-significantly correlated with humidity (table 3). It is also positively and non-significantly correlated with longevity and fecundity (table 2).

3.3 *Pupal period*

The maximum pupal period (12 days) was observed during December and June followed by 11 days from July–November and January (table 1). The shortest pupal period (10 days) was recorded during April, May, February and March. The average pupal period was 10·83 days. This is in confirmation with the range given for tachinids by Clausen (1940).

Pupal period was found to be negatively and highly significantly correlated with temperature and positively and non-significantly correlated with humidity (table 3). It was also positively and significantly correlated with emergence percentage and positively and highly significantly correlated with incubation period and longevity. However, the pupal period was positively and non-significantly correlated with hatching percentage and fecundity (table 2).

Table 1. Data showing seasonal effects on certain developmental stages of uzi fly.

Month	Temp. (°C)	Humidity (%)	Incubation period (h)	Hatching (%)	Pupal period (days)	Emergence percentage	Fecundity	Longevity of ♀ (days)
April 1982	28·9	61·4	30	84·84	10	33·25	269·75	11·50
May	28·4	63·8	30	88·23	10	39·25	290·00	12·25
June	24·7	79·0	60	84·74	12	62·75	561·25	15·75
July	25·4	76·4	48	89·05	11	60·00	1020·50	19·00
August	25·8	80·9	48	90·78	11	82·25	621·75	16·75
September	26·1	71·3	40	89·96	11	78·75	428·75	15·25
October	26·2	68·9	40	84·77	11	82·00	398·25	16·00
November	26·1	66·8	40	89·19	11	71·75	482·25	16·75
December	24·4	56·6	60	87·32	12	59·50	373·00	18·25
January 1983	26·1	53·3	42	88·43	11	79·00	352·50	16·50
February	27·1	55·1	36	77·01	10	51·00	286·50	13·25
March	28·6	50·2	30	83·77	10	37·00	298·50	13·50

	Incubation period	Hatching (%)	Pupal period	Emergence	Fecundity	Longevity
SE ±	NS	NS	NS	1·91	25	0·7
CD at 5%	NS	NS	NS	5·49	71	2·0

Table 2. Data showing the correlation amongst certain developmental stages of uzi fly.

Parameters considered	Correlation coefficient
Pupal period/emergence percentage	+0·5940[a]
Pupal period/incubation period	+0·9458[b]
Pupal period/hatching percentage	+0·3754
Pupal period/fecundity	+0·4101
Emergence percentage/incubation period	+0·4681
Pupal period/longevity	+0·7763[b]
Emergence percentage/hatching percentage	+0·4551
Emergence percentage/fecundity	+0·2772
Emergence percentage/longevity	+0·6697[a]
Incubation period/hatching percentage	+0·2391
Incubation period/fecundity	+0·4938
Incubation period/longevity	+0·7614[b]
Hatching percentage/fecundity	+0·4338
Hatching percentage/longevity	+0·4889
Fecundity/longevity	+0·7096[a]

Levels of significance for correlation coefficient. [a]5%–0·58; [b]1%–0·71.

Table 3. Correlation between temperature, humidity and certain developmental stages of uzi fly.

Parameter	Average pupal period	Average emergence percentage	Average incubation period	Average hatching percentage	Average fecundity	Average longevity
Temperature	−0·9384[b]	−0·6923[a]	−0·9369[b]	−0·3114	−0·5476	−0·8764[b]
Humidity	+0·4360	+0·4610	+0·4489	+0·4666	+0·7053[a]	+0·3821

Levels of significance for correlation coefficient. [a]5%–0·58; [b]1%–0·71.

The pupal period in *Exorista mella* Walker (Diptera: Tachinidae) was reported to be of 27·4 days at 15°C whereas at 30°C it was only 7·8 days (Butler *et al* 1968). This is in confirmation with the present finding.

3.4 *Emergence percentage*

Emergence percentage was maximum during August (82·25), 82% during October, 33·25% during April, average being 61·37 (table 1). Emergence percentage was negatively and significantly correlated with temperature and positively and non-significantly correlated with humidity (table 3). This is in confirmation with the finding of Hefley (1928) on a tachinid *Winthemia quadripustulata* (Fabricius). It was also recorded to be positively and non-significantly correlated with parameters like incubation period, hatching percentage and fecundity whereas it was positively and significantly correlated with longevity (table 2).

3.5 *Longevity*

The average longevity of female flies recorded was 15·39 days. Maximum longevity

(19 days) was recorded during July and minimum (11·50 days) during April (table 1). Sriharan *et al* (1971) reported maximum longevity of female (20 days) in March and minimum (9 days) in October and November. This variation may be due to the difference in environmental conditions.

Longevity was negatively and highly significantly correlated with temperature. However, it was positively and non-significantly correlated with humidity (table 3). This finding is in agreement with the observations on the tachinid parasite *Lespesia archippivora* Riley; the longevity of female was less at higher temperature and more at lower temperature (Bryan *et al* 1969). Even female of *Archytas marmoratus* (Townsend) has the same trend (Patrick 1975).

3.6 *Fecundity*

An average of 448·58 eggs were laid per female fly. Maximum fecundity (1020·50) was in July 1982 and the minimum (269·75) in April (table 1). Fecundity was observed to be positively and significantly correlated with longevity (table 2). It was negatively and non-significantly correlated with temperature and positively and significantly correlated with humidity (table 3). Sriharan *et al* (1971) reported a maximum of 369·50 eggs per female during January and minimum of 69·50 during February and this may be attributed to difference in the experimental environment.

The average number of eggs in the parasite *Euphocera edwardii* (Diptera: Tachinidae) was 190 (Wilkinson and Biever 1974), 310 in case of a tachinid parasite of cabbage looper (Kent and Rabb 1970). In case of *Archytas marmor-atus* the number of eggs laid was more at higher temperature (27°C) than at lower temperature (21°C) (Patrick 1975) which is contradictory to the present finding which can be explained by the finding of Wigglesworth (1953) who states 'the rate of egg production like other processes of metabolism varies with temperature, it is accelerated up to a point and falls rapidly'.

Besides, many other factors are likely to influence the fecundity. These include factors that affect the probability of discovering the host and ovipositing, for example a very low probability of discovering the host would lead to low oviposition. Nutrition of parasitoid larvae and adults also influence fecundity (Bracken 1966; Leius 1961).

The present course of investigation reveals that the effect of temperature and humidity on certain developmental stages of uzi fly are in agreement with the general uniformity recorded in case of several parasites of family tachinidae (Hefley 1928; Clausen 1940; Butler *et al* 1968; Jackson *et al* 1969; Kent and Rabb 1970; Wilkinson and Biever 1974).

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Visual capacity of marine teleost *Nemipterus japonicus* (Bloch)

S RAVEENDRAN and H MD MOHIDEEN
Department of Zoology, The New College, Madras 600 014, India

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Abstract. The differential distribution of cones in the various regions of the retina of the fish *Nemipterus japonicus* (Bloch) was studied. In tangential section two types of cones are observed viz single and double cones, double cones being greater in number than single cones. Of the total cones, double cones constitute 86% while single cones constitute only 14%. In most of the retinal regions, cones are in the square mosaic pattern. The temporal region has greater number of cones than the other retinal regions which were studied. The correlation coefficients for the temporal region with all other regions are invariably positive.

The test shows that there is no significance between the eyes whereas significance is observed among the various retinal regions. The pattern of cone arrangement agrees with the bottom dwelling habit of the fish. The shape of the eye is modified by being slightly oblong with comparatively longer anterio-posterior axis. The retinal cup is more hollow at the temporal region than at the nasal region.

Keywords. *Nemipterus japonicus*; retina; visual cells.

1. Introduction

Retina is a sheet of photoreceptors connected to the brain via the optic nerve. Greef (1900) distinguished different types of cones in the retinas of fishes. Muller (1952) and Lyall (1957) studied the cone mosaics in some teleost fishes. Engstrom (1963) reviewed the ecology and phylogeny of teleostean visual cells. Changes in square mosaic pattern to row mosaic pattern in dark adaptation have been noticed recently by Kunz (1980). Munro (1984) suggested the origin of double cones from single cones as the result of their incomplete fission. O'Connell (1963), Tamura and Wisby (1963) and Feldman and Carleton (1984) studied the structure of visual cells and their distribution in the retinas of teleosts based on their tangential sections. Siminoff (1984) studied trichromatic cones in vertebrate retina viz red, green and blue cones. The movement of pigments in the epithelial pigment layer causes photochemical changes. The contraction and retraction of cones and rods depend upon the ambient light levels (Prosser 1973).

Yamanouchi (1940) measured the density of cones in 5 regions of the retina of *Microcanthus strigatus* and obtained the highest density in the temporal region. Kahmann (1934, 1936) described the fovea centralis in *Serranus julus* and *Blennius* and showed that it is most often located in the temporal region. Electrical stimulus also has chemical effect on the release of many peptides affecting the epidermal chromatophores and this has been illustrated by the experiment conducted on isolated eye stalks of *Uca pugilator* (Bush *et al* 1984).

Extensive investigations have been carried out on the diverse aspects of fish vision. However studies concerning histometry of fish eye are very limited. The present work is aimed at studying the cone type, cone mosaic pattern and cone density in the different regions of the eye of *Nemipterus japonicus* and to find out as to how they aid the visual perception of this fish.

2. Materials and methods

For the present study *N. japonicus*, (FAO 1974), a teleost fish belonging to the family Nemipteridae, was selected for investigating the retinal structure. The histological details of cones in the retinas are elucidated only in the tangential sections. The retinal cup was cut into 8 equal pieces, viz temporal, dorso-temporal, dorsal, dorso-nasal, nasal, ventro-nasal, ventral and ventro-temporal (figure 17). Each retinal bit was tied between two cover slips separately to flatten them. They were embedded in paraffin and 2 μ thick tangential sections of these bits of retina were cut, stained with hematoxylin and counterstained in alcoholic eosin. The single and double cones were counted separately in right and left eye (tables 1 and 2).

2.1 *Statistical methods*

2.1a *Correlation co-efficient*

Correlation co-efficient between 8 regions of the right eye and the respective regions of the left eye with regard to double cones, single cones and total cones were carried out (tables 3 and 4).

Table 1. Patterns of cone arrangements with ratio between double and single cones.

Functional regions selected	Pattern of cone arrangement observed	Ratio between double and single cones	Figure nos
Temporal	Square mosaic	7:1	1, 9
Ventro-temporal	Cone mosaic	7:1	2, 10
Dorso-temporal	Row and square mosaic	8:1	3, 11
Ventral	Square mosaic	8:1	4, 12
Dorsal	Square mosaic	8:1	5, 13
Nasal	Square mosaic	9:1	6, 14
Dorso-nasal	Square mosaic	8:1	7, 15
Ventro-nasal	Square mosaic	9:1	8, 16

Table 2. Average numbers of cones in 0·16 mm^2 in various regions of retina in *N. japonicus* right and left eye.

Retinal regions	Right eye		Left eye	
	Double cones	Single cones	Double cones	Single cones
Temporal	163	32	139	58
Dorso-temporal	109	16	150	23
Dorsal	70	8	96	6
Dorso-nasal	92	12	42	5
Nasal	78	10	70	7
Ventro-nasal	67	12	57	5
Ventral	93	12	85	9
Ventro-temporal	166	23	157	22

Table 3. Correlation co-efficient between the right and left eyes.

Double cones	Single cones	Total cones
0·7791	0·9476	0·8494

Table 4. Correlation co-efficient between total number of cones in the entire retinal region (right and left) to total number of cones in each retinal region (right and left).

	Temporal	Dorso-temporal	Dorsal	Dorso-nasal	Nasal	Ventro-nasal	Ventral	Ventro-temporal
cones								
cones	0·8035	0·4197	0·4027	0·3272	0·7578	0·2048	0·8978	0·2372
ones	0·7632	0·1816	−0·3839	−0·4177	−0·0600	−0·0821	0·3192	0·6472
nes	0·7836	0·3691	0·4669	0·2309	0·6668	0·2671	0·8171	0·1627

Table 5. Anova table

Type of cones	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	Variance ratio F
Double cones	Eyes	1	110·25	110·250	0·2795
	Region	7	21672·75	3096·107	7·8503*
	Error	7	2760·75	394·392	
Single cones	Eyes	1	6·25	6·25	0·1049
	Region	7	2350·00	335·7142	5·6388*
	Error	7	416·75	59·5357	
Total cones	Eyes	1	64·00	64·00	0·135021
	Region	7	35697·75	5099·67	10·758815*
	Error	7	3318·00	474·00	

*Significant difference is observed at 5% level ($P < 0·05$).

Significance of variation

cance of variation was tested between the eyes and between the regions for of the cone type using the analysis of variance technique by two way cation (table 5).

sults

etina may vary from species to species in its thickness, cone density and ns of arrangement of cones. Within the retina the cone density may vary from to region. In the present study 8 different regions of the retina of the right and e of *N. japonicus* were histologically studied and the photomicrographs show e and arrangement of cones in the various regions (table 1).

Temporal region (figures 1 *and* 9)

one density is high and also the size of the cone is bigger in this region than in her regions, in both right and left eyes. The double cones are regularly arranged and single cones are irregularly arranged. About 20% of the retinal cones are located in the temporal region. The optic nerve originates in this region. Square mosaic patterns are observed here.

3.2 *Ventral-temporal* (*figures* 2 *and* 10)

In this region for every 7 double cones one single cone is present. The row mosaic pattern is observed.

3.3 *Dorso-temporal* (*figures* 3 *and* 11)

Here for every 8 double cones one single cone is present. Both row and square mosaic patterns are observed.

3.4 *Ventral* (*figures* 4 *and* 12)

For every 8 double cones one single cone is present. The square mosaic pattern is observed.

3.5 *Dorsal* (*figures* 5 *and* 13)

The single cones are least in number here compared to the rest of the retina the cones being arranged in a scattered manner. Square mosaic patterns are observed here.

3.6 *Nasal* (*figures* 6 *and* 14)

For every 8 double cones one single cone is present in the right eye and for every 10 double cones one single cone is present in the left eye. Square mosaic patterns are observed here.

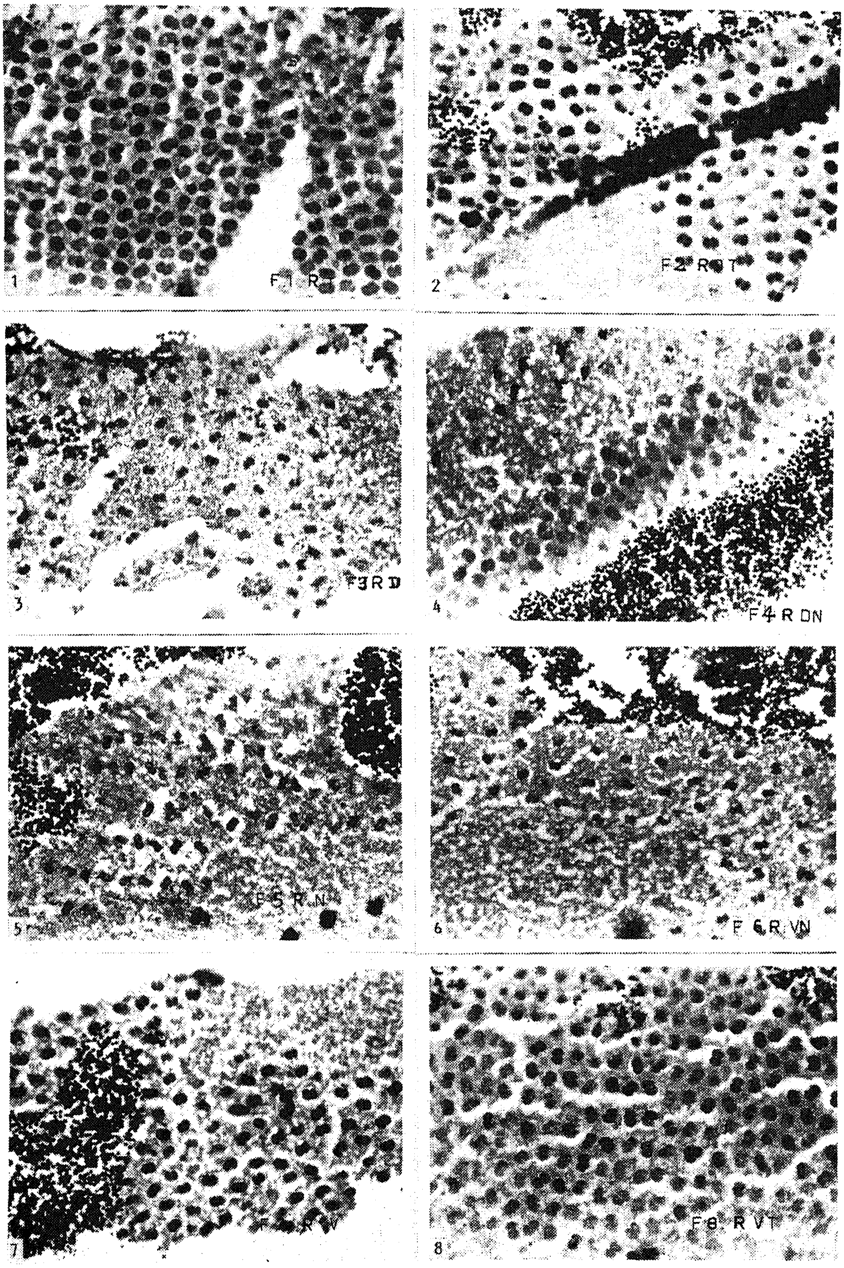
3.7 *Dorso-nasal* (*figures* 7 *and* 15)

For every 8 double cones, one single cone is present. The cones are small in size. Square mosaic patterns are observed here.

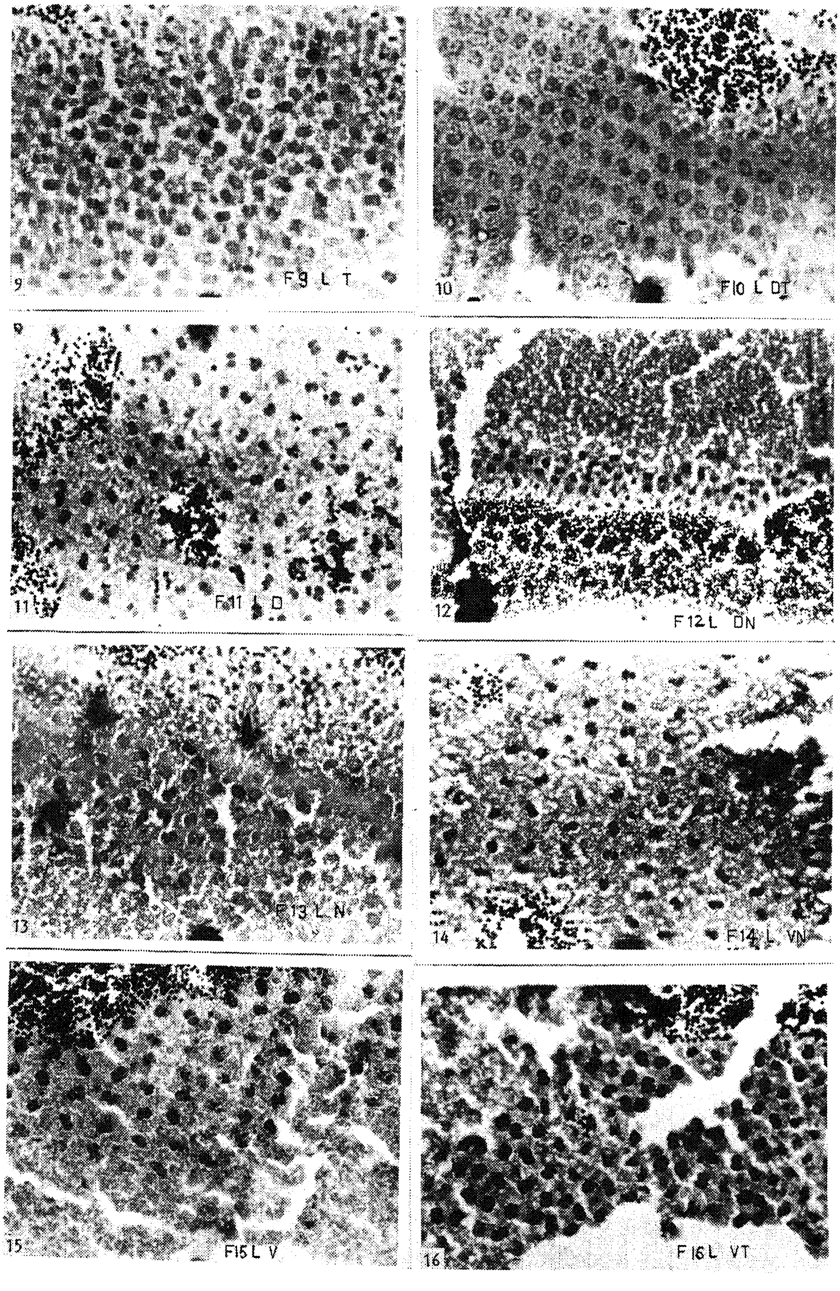
3.8 *Ventral-nasal* (*figures* 8 *and* 16)

This retinal region possesses the least number of cones compared to the other retinal regions. Here also for every 8 or 9 double cones one single cone is present. Square mosaic patterns are observed here.

Correlation co-efficient studies obviously indicate positive correlation between total cones and the number of cones in the different regions. However r values differ for different regions (table 4). Highest positive correlation is shown between total number of cones in the ventral region and the total number of cones in the retinal region (r 0·8171). Correlation co-efficient is least between total number of cones in the ventro-temporal region and the total of cones in the retinal region (r 0·1627).



Figures 1–8. Photomicrographs of tangential sections of the retinal areas of *N. japonicus* showing cone density and cone mosaic patterns. **1.** Right temporal (RT). **2.** Right dorso-temporal (RDT). **3.** Right dorsal (RD). **4.** Right dorso-nasal (RDN). **5.** Right nasal (RN). **6.** Right ventro-nasal (RVN). **7.** Right ventral (RV). **8.** Right ventro-temporal (RVT).



Figures 9–16. Photomicrographs of tangential sections of the retinal areas of *N. japonicus* showing cone density and cone mosaic patterns. **9.** Left temporal (LT). **10.** Left dorso-temporal (LDT). **11.** Left dorsal (LD). **12.** Left dorso-nasal (LDN). **13.** Left nasal (LN). **14.** Left ventro-nasal (LVN). **15.** Left ventral (LV). **16.** Left ventro-temporal (LVT).

Correlation co-efficient between the total number of cones and the total number of double cones in the different regions indicates almost similar pattern when compared to that of the total number of cones in their different regions. However, comparison of correlation co-efficient between the total number of single cones and single cones in different regions shows marked difference with regard to *r* values. Temporal, dorso-temporal, ventral and ventro-temporal regions show positive correlation having *r* values of 0·7632, 0·1816, 0·3192 and 0·6472, respectively (table 4). However, dorsal, dorso-nasal, nasal and ventral-nasal regions show negative correlation having *r* values of −0·3839, −0·4177, −0·0600 and −0·0821, respectively (table 4).

Correlation co-efficient studies between right and left eye with regard to the double cones, single cones and total cones show positive correlation (table 3). The single cone shows higher *r* value (0·9476) when compared to the total cone *r* value (0·8491) and double cone *r* value (0·7791).

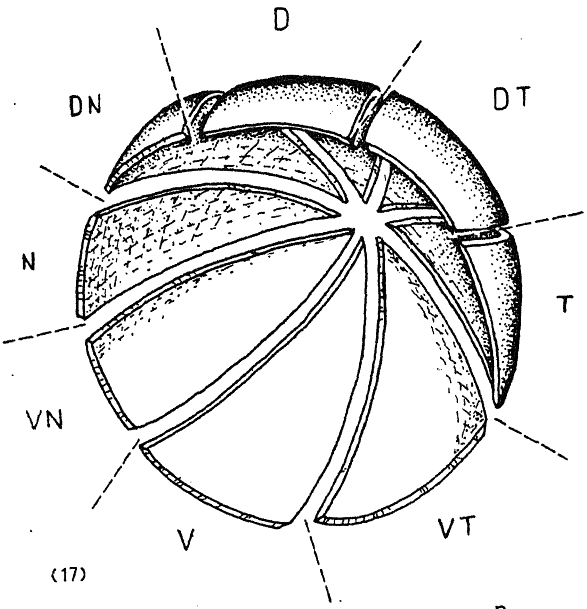
Analysis of variance between right and left eye and within the retinal regions to find out any significance at 5% level clearly indicates that there is no significance among the right and left eye. However, significance is observed among the different regions of the right and left eye. Similar results are obtained from the analysis of variance between the double and single cones of different regions of right and left eyes (table 5).

The actual numbers of single, double and total cones are drawn in topographical diagrams which also indicate the cone density (figures 17–23).

4. Discussion

In the retina the cone density may vary from species to species. Sometimes the cone density may vary from region to region according to the regional function. Generally two types of cones are observed; double cone and single cone. Furst (1904) distinguished difference among various kinds of single cones in *Salmo*. The cone present at the centre of the square mosaic was named by him as 'Mittelzapfen' and cones present in the corners as 'Zwischenzapfen'. From the studies of Muller (1952) and Lyall (1957) it is known that the central single cones are long and the single cones occupying other positions in the mosaics are short or they are additional single cones (Engstrom 1963). The presence or absence of the single cones depends upon the functional aspect of the retina (Tamura 1957).

During the 19th century terms such as twin cones 'Zwillingzapfen' or 'Zwillinge' were common without any distinction between equal and unequal double cone types and thus these reports only tell us that some sort of double cone elements are present. Greef (1900) distinguished between twin cones (Zwillingzapfen) and double cones (Doppelzapfen). Munro (1984) observed that in *Acquidens portalegrensis* the structural components of the adult retina are present within 7 days after fertilization. Double cones initially appear a day earlier and then originate from the incomplete fission of single cones. The present work on *N. japonicus* shows equal and unequal double cones as observed in the retina in the Poecilidae (Engstrom 1960). The number of cones also varies considerably between different regions of the retina and it is quite obvious that there is variation in the shape of the cones. The present work shows that the cone density is higher in the temporal region where the number of cones is larger and their size is bigger than in the other retinal regions. The optic nerve also enters in temporal region. Yamanouchi (1940) measured the density of



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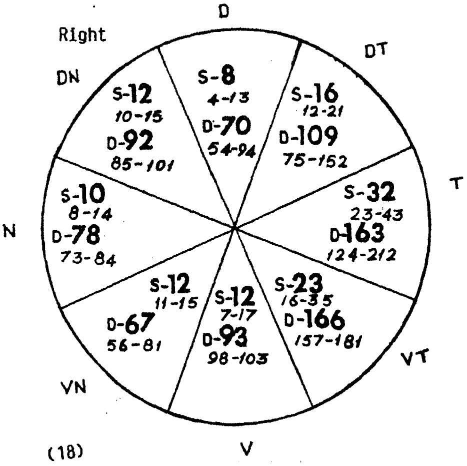

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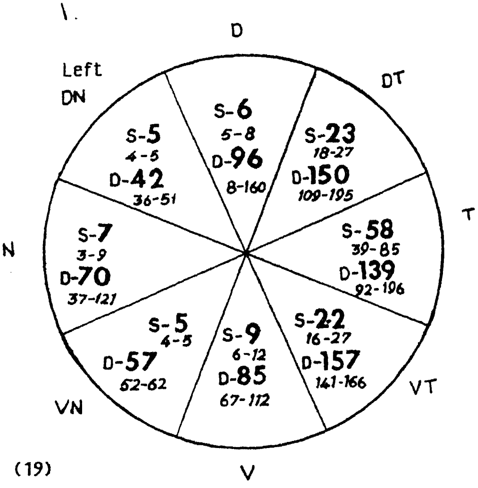

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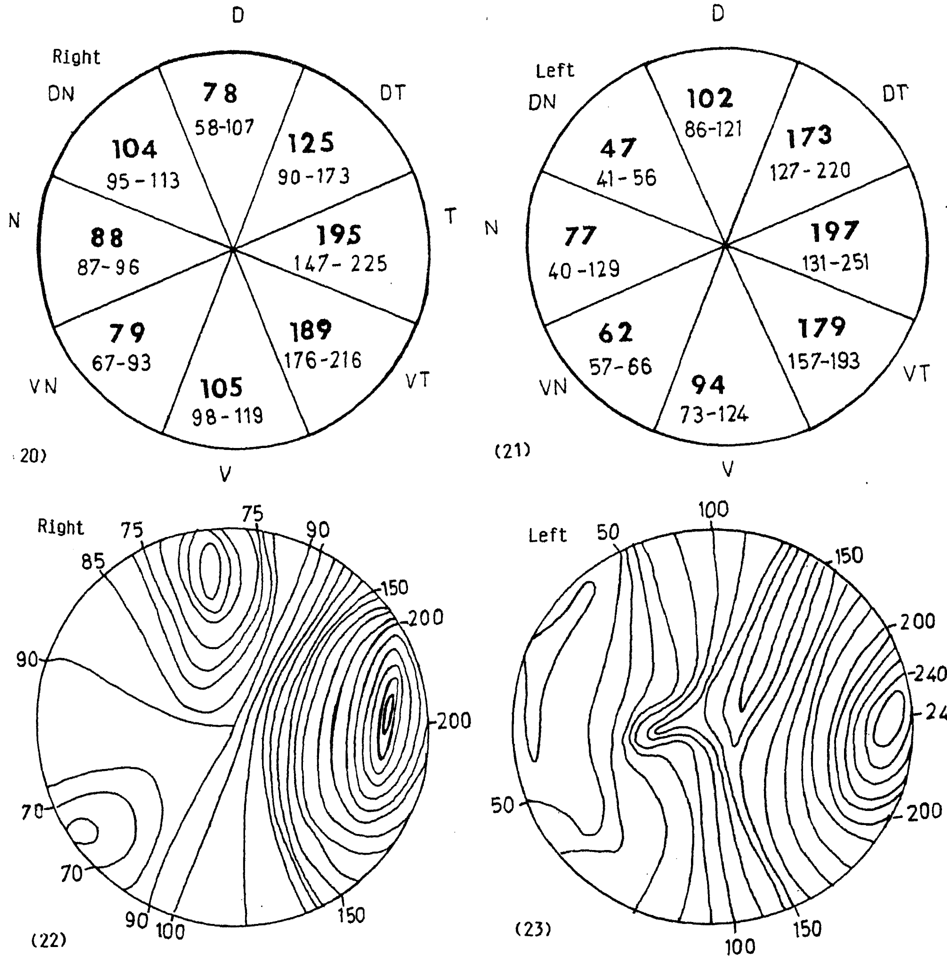
Figures 17–19. 17. Perspective drawing of the retina of *N. japonicus* showing the orientation of tangential section preparation. **18 and 19.** The density of single cones and double cones in 8 segments of the retina of *N. japonicus*. The means are shown in large types, the ranges in small types. **18.** Right eye. **19.** Left eye.

cones in 5 regions of the retina of *Microcanthus strigatus* and observed the highest density in the temporal region. Kahmann (1934, 1936) described the fovea centralis in certain fishes and showed that it is most often located in the temporal region (*Serranus julus* and *Blennius*). Tamura and Wisby (1963) observed the same in the greater amberjack and dolphin.

In *N. japonicus* the number of double cones is greater than that of the single cones, with both the right and left eyes consisting of 86% of double cones and 14% of single cones. *N. japonicus* lives generally in 40 metres depth and rarely at or below 60 metres (FAO 1974). It is a bottom dweller which possesses higher number of double



Figures 20–23. 20 and 21. The density of total cones in 8 segments of the retina of *N. japonicus*. The means are shown in large types, the ranges in small types. **20.** Right eye. **21.** Left eye. **22 and 23.** The probable total cone topography of the retina. **22.** Right eye. **23.** Left eye.

(D, Dorsal; DN, dorso-nasal; DT, dorso-temporal; N, nasal; T, temporal; V, ventral; VN, ventro-nasal; VT, ventro-temporal).

cones. The double cones react in lower light intensities than the single cones. So the double cones are greater in number than the single cones in this species. Tamura (1957) concluded that the abundance of single and twin cones depend on the depth of habitation of the fish. *Scomber* which lives on the surface has almost equal number of twin and single cones and *Helicolenus malakichthys* which lives in 100–300 metres depth did not have single cones and had twin cones only in the ventral and ventro-temporal regions (Tamura 1957). *N. japonicus* occupies an intermediate depth between the above two species i.e. 40 metres depth. So its possession of double cones and single cones 86% and 14%, respectively is obviously in conformity with its habitat.

N. japonicus often selects the sand burrowing preys such as polychaetes, crabs, squilla etc (Vivekanandan, unpublished results) living at the bottom for which this fish needs acute vision. In such a case the temporal region is very much helpful having 20% of the total cones in this region (an *r* value of 0·7836 with total retinal cones). This is followed by the dorso-temporal region (16% of cones with *r* 0·3691).

An observation in the distribution of the number of cones in the eye prompts the division of the eye into two functional areas. Temporal, dorso-temporal and ventro-temporal acuity as one functional area and the rest of the retinal acuity as a second functional area, the former possessing about 175 cones (double and single) and the latter 83 cones (double and single). The functional area 1 has more than double the number of cones present in the functional area 2. By observing its habitat it is predicted that the average number of cones represented by functional area 2, if also present in functional area 1 may be sufficient for swimming. The niche, particularly the trophic niche, of this fish demands an extensive development of the temporal area, probably an area temporalis as suggested by Tamura (1957). This fish being a bottom feeder, shows structural modifications of buccal and nasal regions suitable to its feeding habitats. The eyes also fall in line with the modification by being slightly oblong, having comparatively long anterior-posterior axis. The cup is hollow at the temporal region compared to anterionasal region. Correlation being positive within the temporal region also confirms this statement. The anterior tapering of rostral region with a terminal mouth facilitates viewing while feeding. No doubt the bottom layer would certainly be dimmer compared to pelagic zones. Depending on the depths and clarity of waters, the soil/sand-water interface would fall under photic or aphotic conditions. These conditions demand greater acuity of vision at the temporal region of the eye. Thus the temporal region of the eye including the dorso-temporal and the ventro-temporal regions will act as an additional function zone with reference to feeding specialization which accounts for the increased number of cones in this area.

The *r* values for single cones in the ventral-temporal and ventral regions viz 0·6472 and 0·3192, show that this species is also able to observe moving prey at the surface level. It preys upon small fishes and moving prey. This posterior region of the retina has greater number of cones than the anterior retinal region. This species does not continuously live at 40 metres depth; it may at times come to the surface, during which time the single cones in all these regions may help to observe the prey as well as to escape from the enemies, the other receptor systems also helping in the process.

The presence of single cones and twin cones in teleost retina and the arrangement of both these types of cones together form a mosaic pattern. In general row mosaic, square mosaic and non-regular mosaics types are common among the teleosts and are to be related to the ecology as also to the phylogeny of the fish (Lyall 1957; Engstrom 1963). The row mosaic is primitive and the square mosaic is assumed to have developed from row mosaic (Muller 1952; Lyall 1957). The square mosaic pattern of retinal cones changes during dark adaptation into a row mosaic in the guppy, *Poecilla reticulata* Kunz (1980). In the present work on *N. japonicus* most of the regions have square mosaic patterns which show that it belongs to one of the advanced type of fishes.

When the total number of cones viz double, single and total are correlated with individual retinal regional double, single and total cones, the results show that the temporal region is highly correlated to that of the total number of cones (*r* values being 0·8035, 0·7632, 0·7836 for double, single and total cones, respectively (table 4).

The total number of cones in the temporal region is more than in the other retinal regions. The ventral region also shows higher values of *r* 0·8978, 0·3192, 0·8171 for double, single and total cones, respectively (table 4) enabling this fish to survive also in surface regions. A correlation between the total single cones of different areas due to regional specialization shows a negative correlation in the anterior portion of the retina and positive correlation in the rest of the posterior part of the retina (table 4).

Analysis of variance at 5% of significance (table 5) shows no significance between the right and left eye taking into account double, single and total cones and their total number. However taking the different cones viz double, single and total cones and their total number into consideration, regionwise, they show significant variations from one another in accordance with their function.

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ne and energy budgets of alfalfa pollinating bees *Megachile nana* ıgh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae)

D P ABROL*
Department of Zoology, Haryana Agricultural University, Hisar 125 004, India
*Present address: Division of Entomology, S K University of Agricultural Sciences and Technology, Srinagar 191 121, India

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Abstract. Laboratory measurements of physiological energetics were coupled with the field observations on time budget/energy intake of *Megachile nana* and *Megachile flavipes* to estimate the daily energy budget. The energy budget varied from one day to another, and crop to crop. In general, the foraging profitability/attractibility was in the following order for both the species: *Parkinsonia aculeata* > *Medicago sativa* > *Trifolium alexandrium.* The studies revealed the competitive superiority of *Parkinsonia aculeata* over *Medicago sativa* and *Trifolium alexandrium.*

Keywords. Energy budget; alfalfa; *Megachile nana*; *Megachile flavipes*; pollination.

Introduction

ıajor challenge facing an animal is the adaptive allocation of time and energy ɔng its various daily activities associated with the self maintenance and roduction. For ecological studies concerned with the optimization of time and rgy budgets the division of efforts among various activities must be known. This essitates first construction of time budgets and then conversion to appropriate rgetic costs. The time and energy budgets of the organisms are quite variable, sumably in relation to varied ecological conditions and physiological costs. Since siological processes such as metabolic costs, clearly are under short term control he physical environment. The temporal constrains on control of the distribution ime and energy into various activities are not equally obvious. Most studies of e/energy budgeting have focussed on birds, especially nectarivores (Stiles 1971; lf and Hainsworth 1971; Gill and Wolf 1975) because (i) they are usually easy to erve, (ii) their activities are classifiable into discrete categories (e.g. flight, rest and ɔor) and (iii) their energy intake is rather easily quantified. Because of their ividual nesting habit the solitary bees share many of the similar properties, and vide an excellent workable system for this type of investigation. They can be ly monitored, and categories of behaviour discerned and timed. This paper orts on the time-energy budgets of two alfalfa pollinating bees *Megachile nana* gh and *M. flavipes* Spinola, and concentrates on the apparent relationship veen the energy intake from the environment and the time/energy budgeting tern of these bees.

Materials and methods

time-energy budgets of *M. flavipes* and *M. nana* were studied in relation to *culeata*, *M. sativa* and *T. alexandrium.* To determine differential attractibility and rgy budget balance, the following studies were conducted.

2.1 *Sampling of bee population*

The relative abundance of bees was assessed by the method suggested by Linsley *et al* (1952) with some modifications to suit the present investigations. For this purpose, 5 plots each measuring 1 m^2 were selected and bees were counted for 30 s from each side of the plot.

2.2 *Determination of caloric reward/flower/day*

Inflorescences were bagged with the nylon mesh to exclude all the nectarivores. The nectar from the flowers was sampled with the help of microcapillary pipettes at an interval of 2 h throughout the day. The volume of nectar obtained was measured with the help of 5 λ pipettes and its sugar concentration determined with a hand refractometer. Caloric reward/flower/day was calculated following Heinrich (1975) that 1 mg of sugar irrespective of the type yields 16·74 joules of energy.

2.3 *Rate of foraging/nectar extraction*

The time spent by a bee gathering nectar was recorded using a chronometer with an accuracy of 0·01 s. The number of flowers visited by an individual and time taken to do so was recorded by following their movements as long as possible. Accordingly the number of flowers visited per minute was calculated.

2.4 *Determination of time budgets*

The time budgets of the marked bees were obtained by watching their activities right from commencement to cessation at the experimental nesting sites of the Department of Zoology. All activities were timed with a stop-watch to the nearest of 0·5 s. For data analysis the timed observations were divided into 3 discrete categories.

2.4a *Flying or foraging*: This was the total time the bees spent for collecting nectar, pollen, mud and leaves etc.

2.4b *Activity*: This included the time spent in nest after returning from foraging trips. During this period, the bee remained active and unloaded the pollen mass.

2.4c *Resting*: This period was taken when the activity of the bees ceased and did not come out.

2.5 *Metabolic measurements*

The metabolic expenditures specific to each type of activity (i.e. rest, flight and activity) and temperature were obtained from the regression equations (table 1). The oxygen consumption determinations were made in rest, flight and activity following

Table 1. Respiration rate regressions (temperature vs oxygen consumption).

Species	State of activity	Regression equation	N	Syx
M. nana	Flight	$Y = -31{\cdot}74 + 2{\cdot}556x$	33	$\pm 1{\cdot}980$
	Activity	$-1{\cdot}214 + 0{\cdot}06692x$	35	$\pm 0{\cdot}039$
	Resting	$-0{\cdot}421 + 0{\cdot}03616x$	32	$\pm 0{\cdot}091$
M. flavipes	Flight	$-49{\cdot}13 + 2{\cdot}916x$	33	$\pm 3{\cdot}410$
	Activity	$-0{\cdot}5280 + 0{\cdot}03116x$	38	$\pm 0{\cdot}029$
	Resting	$-0{\cdot}5638 + 0{\cdot}0296x$	33	$\pm 0{\cdot}004$

Abrol (1986) at temperatures 20°, 25°, 30°, 35° and 40°C within which foraging occurs, using constant volume Warburg respirometers. The specific procedure followed in each case is detailed below:

2.5a *Determination of oxygen consumption during flight*: Experiments were conducted during day time in complete darkness so that even in day time if bees are exposed to flickering flashes of light, they get stimulated and initiate flight. Flickering flashes were maintained by connecting 100 watt lamp with the circuit of rotating drum of kymographic apparatus which automatically gave 640 flickering flashes/minute.

2.5b *Determination of oxygen consumption during active state*: Bees were collected at peak activity hours from the field. The lag period between collection and measurement of oxygen consumption approximately never exceeded more than one hour.

2.5c *Determination of oxygen consumption during resting state*: Bees are diurnal and their activity remains suspended during night which constitutes their resting period. The respiratory measurements were made during the night time and bees used were collected from the field in the evening and kept in darkness prior to the start of the experiment. The bees were introduced into the Warburg flasks with blunt forceps under red light to which bees are insensitive (i.e. blind). The observations from the respiroscope were recorded using spot light so that the bees may not get disturbed.

The rate of oxygen consumption was calculated on unit weight basis. The oxygen consumption values were converted to energy values by considering that 1 ml of oxygen consumption is equivalent to 20·93 joules of energy expenditure irrespective of the substrate utilized (Heinrich 1975).

2.6 *Evaluation of time-energy budget balance*

The data were computed following Pyke (1979) with some modifications to suit the present investigations. To compute the time-energy budget balance following variables were defined

T = Total available time for various activities (i.e. 24 h).
Tf = Total foraging time/day.
Ta = Total active time/day.
Tr = Total resting time/day.

Cf = Rate of energy expenditure while foraging (joule/day).
Ca = Rate of energy expenditure while active (joule/day).
Cr = Rate of energy expenditure while resting (joule/day).

Thus it follows that

$$T = Tf + Ta + Tr \tag{1}$$

and

$$C = Cf \times Tf + Ca \times Ta + Cr \times Tr \tag{2}$$

Where C = total energetic cost (joule/day).

Gross energetic gain of the bees is given by

$$G = \mu r\,Tf. \tag{3}$$

Where r = the rate at which a bee visits flowers for nectar while foraging.
μ = Standing crop of nectar (average energy/flower/day).
Tf = Total foraging time.

Thus by substracting (2) from (3) we get

$$E = \mu r Tf - Cf\,Tf + CaTa + CrTr. \tag{4}$$

Where E = net energy balance (joule/day).

3. Results

The data in table 2 show the time-energy budgeting pattern of *M. nana*. It was found that *M. nana* on an average spent 184 min in foraging (nectar pollen etc.), 194·40 min

Table 2. Time-energy budget of *M. nana*/day.

	Proportion of time spent (min)			Average temperature (°C)	
Date of observation	Foraging (Tf)	Active (Ta)	Resting (Tr)	Day	Night
April 1984					
15	163	217	1060·00	30·30	21·60
17	181	205	1054·00	31·00	22·00
24	202	222	1016·00	31·20	22·30
26	180	183	1077·00	33·50	21·50
28	194	170	1076·00	33·00	21·40
Average	184	199·40	1056·40	31·80	21·76

Energy budget of *M. nana*/day (average weight of bee = 27·17 mg, n = 90).

Proportion of energy expenditures (joule/min/bee)

Foraging (Cf)	Active (Ca)	Resting (Cr)
0·4692	0·0086	0·0034

Energy expenditure/bee/day $C = Cf \times Tf + Ca \times Ta + Cr \times Tr$
$= 0{\cdot}4692 \times 184{\cdot}00 + 0{\cdot}0086 \times 199{\cdot}40 + 0{\cdot}0034 \times 1056{\cdot}40 = 91{\cdot}63$ joule.
Thus $C = 91{\cdot}63$ joule/bee/day

Note: (i) Calculation based on combined time budget figure.
(ii) Calculation based for each category of the time budget at the temperatures indicated.

in nesting/active state and 1056·40 min in resting/day. The energy expenditures at the temperatures 31·8°C (foraging and activity) and 21·76°C (resting) as determined from the rate of oxygen consumption was found to be 91·63 joule/bee/day. However, *M. flavipes* under similar conditions spent 216·00 min in foraging, 233·60 min in activity/nesting and 990·40 min in resting. The energy expenditure was found to be 146·38 joule/bee/day (table 3).

The data on the energy rewards offered by their host plants and the time spent in extracting these rewards for both the species are presented in table 4. In general, *P. aculeata* produced 2·90 ± 0·45 joule/flower/day, *M. sativa* 1·20 ± 0·12 joule/flower/day while *T. alexandrium* produced 0·393 ± 0·057 joule/flower/day. The

Table 3. Time-energy budget of *M. flavipes*/day.

	Proportion of time spent (min)			Average temperature (°C)	
Date of observation	Foraging (Tf)	Active (Ta)	Resting (Tr)	Day	Night
April 1984					
15	188·00	247·00	1005	30·30	21·60
17	198·00	218·00	1024	31·00	22·00
24	222·00	249·00	969	31·20	22·30
26	243·00	214·00	982	33·50	21·50
28	229·00	239·00	972	33·00	21·40
Average	216·00	233·60	990·4	31·80	21·76

Energy budget of *M. flavipes*/day (average weight of bee = 43·72 mg, n = 90).

Proportion of energy expenditure (joule/min/bee)

Foraging (Cf)	Active (Ca)	Resting (Cr)
0·6647	0·00704	0·00118

Energy expenditure/bee/day $C = Cf \times Tf + Ca \times Ta + Cr \times Tr$

$= 0{\cdot}6647 \times 216{\cdot}00 + 0{\cdot}00704 \times 233{\cdot}60 + 0{\cdot}00118 \times 990{\cdot}40 = 146{\cdot}38$ joule.

Thus $C = 146{\cdot}38$ joule/bee/day.

Note: (i) Calculation based on combined time budget figure.
(ii) Calculation based for each category of the time budget at the temperatures indicated

Table 4. Energy reward/flower/day and number of flowers visited/min by *M. flavipes* and *M. nana*.

		Flowers visited/min[b]	
Species	Joule/flower/day[a]	*M. flavipes*	*M. nana*
M. sativa L.	1·20 ± 0·128 (1·028 – 1·411)	12·55 ± 1·83 (10·52 – 15·20)	8·55 ± 2·91 (6·52 – 11·30)
P. aculeata	2·90 ± 0·459 (2·140 – 3·328)	9·07 ± 2·74 (6·92 – 12·32)	5·40 ± 0·89 (3·45 – 6·80)
T. alexandrium	0·393 ± 0·057 (0·3110 – 0·4759)	16·89 ± 2·35 (14·81 – 19·87)	12·42 ± 3·09 (8·45 – 15·41)

[a] Mean ± SD of 200 observations. [b] Mean ± SD of 35 observations.

rate of extraction of caloric rewards also varied from crop to crop and species to species. *M. flavipes* on an average visited 9·07 ± 2·74 flowers of *P. aculeata*, 12·55 ± 1·83 flowers of *M. sativa* and 16·89 ± 2·35 flowers of *T. alexandrium*/min while this rate was found to be 5·40 ± 0·89, 8·55 ± 2·91 and 12·42 ± 3·09, respectively for *M. nana*.

The energy budget resulting from the energy intake and expenditure is presented in table 5. The results highlighted that *P. aculeata* was most profitable as the bees could ensure a greater reward than the other two crops. The foraging profitability was in the following order: *P. aculeata* > *M. sativa* > *T. alexandrium*. The attractibility of the bees, as evidenced by their foraging population on these crops, also exhibited the same pattern (table 6). The studies revealed that in competition for pollinators *P. aculeata* had an edge over *M. sativa*, while the latter was preferred than *T. alexandrium*. It is thus clearly evident that in competition with *P. aculeata* the other two crops are at disadvantage.

4. Discussion

Energy requirement of the bees and caloric rewards offered by the flowers determine whether or not an animal can be a dependable flower visitor (Heinrich and Raven 1972; Heinrich 1975). Rewarding system developed by flowers enable bees to make distinction between them and closely related species or ecotypes. This has resulted in

Table 5. Energy intake, expenditure and budget balance.

Bee species	Plant species	Energetic gain (joule)	Energetic cost (joule)	Energy balance (joule)
M. nana	*P. aculeata*	2843·63	91·63	2752·00
	M. sativa	1630·02	91·63	1538·39
	T. alexandrium	716·97	91·63	625·34
M. flavipes	*P. aculeata*	1442·20	146·38	1295·82
	M. sativa	945·99	146·38	799·61
	T. alexandrium	449·11	146·38	302·73

Note: Since solitary bees collected both nectar and pollen in a foraging trip therefore, to minimize error foraging time was halved for calculation of energy intake.

Table 6. Comparative abundance of bees.

		Abundance of bees ($5\ \text{m}^2$)		
Bee species	Hour of observation	*P. aculeata*[a]	*M. sativa*[a]	*T. alexandrium*[a]
M. flavipes	10:00	35·30 ± 5·15	23·50 ± 3·80	7·50 ± 2·15
	20:00	44·40 ± 4·80	20·10 ± 2·79	13·80 ± 2·19
	14:00	30·80 ± 4·79	15·70 ± 1·85	10·40 ± 1·79
	16:00	12·70 ± 2·70	5·20 ± 3·15	—
M. nana	10:00	27·30 ± 3·90	12·90 ± 2·59	5·30 ± 1·30
	12:00	35·80 ± 4·10	15·20 ± 3·11	10·20 ± 1·97
	14:00	38·10 ± 3·75	13·20 ± 2·17	11·40 ± 1·87
	16:00	8·30 ± 1·80	5·80 ± 1·70	1·33 ± 0·40

—indicates no bee activity. [a]Mean ± SD of 5 observations.

a mutualistic development of a co-evolutionary process and have evolved co-partnership between the two (Leppik 1977). The interdependence of pollinating bees with flowers depend much upon their energy requirement and the balance sheet they share with flowers (Heinrich and Raven 1972). The pollinators with high energy requirements may not forage at the flowers, which provide low caloric rewards (Heinrich 1983). *P. aculeata* provided the maximum caloric reward/flower/day followed by *M. sativa* and *T. alexandrium*. The former crop/plant attracted the bees in large numbers followed by the latter two crops. This differential attractibility was probably due to the caloric rewards. *M. sativa* was intermediate in attracting foraging population and had an competitive edge over *T. alexandrium*. The bees visited *M. sativa* during early hours of the day, thereafter the foraging population shifted to *T. alexandrium*. This was probably due to the depletion of nectar in *M. sativa* which compelled the foraging population to visit even low rewarding flowers of *T. alexandrium*. Similar results were obtained by Heinrich (1975) who reported that the bees may visit low rewarding flowers, when the nearby resources are depleted or become sparse. Jain and Kapil (1980) reported that *P. aculeata* had an edge over *M. sativa* in attracting foraging population of these bees. They reasoned that presence of maltose component in nectar of *P. aculeata* (not present in *M. sativa* and *T. alexandrium*) was the main cause of attracting large number of bees. The present investigations however do not support their contention, since according to Rowley (1976), there is no biochemical pathway for the synthesis of maltose in nectar yielding plants, wherever it is present, arises as a bacterial/fungal contaminant and/or due to the glucophilic enzymes introduced by the insects. Therefore, to assign the attractibility to this foreign element seems to be inappropriate. In the present investigations, besides the physical factors the attraction of bees was attributed to the caloric rewards offered by the flowers and foraging profitability resulting there from. This view is also supported by their comparative abundance on these crops under similar conditions (table 6).

The above studies examine some of the features of the economical crop plants competing for pollinators, however, future research is needed on related aspects like (i) factors effecting yield of nectar in *M. sativa*, (ii) genetic manipulations and selection of varieties with attractive petals, (iii) to study the pests and parasites of bees, and (iv) to increase their population through management practices. These studies if carefully undertaken are likely to generate better understanding for utilization of these bees as perspective pollinators.

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Induction and genotypic verification of recombinants in males of *Drosophila melanogaster*

GURBACHAN S MIGLANI and VINDHYA MOHINDRA
Department of Genetics, Punjab Agricultural University, Ludhiana 141 004, India

MS received 20 August 1985

Abstract. Ethyl methanesulphonate (0·75%) was mixed with food (1:9) and fed to developing F_1 (Oregon-K +/*dumpy black cinnabar, dp b cn*) individuals in second one-third part (54–86 h after egg deposition) of larval life, at 25 ± 1°C, for inducing recombination in males of *Drosophila melanogaster*. In control and 0·75% ethyl methanesulphonate experiments, pooled test cross progeny comprised 3475 and 13887 flies, respectively, out of which 9 and 236 were recombinants. From these, 9 and 192 recombinants were further test-crossed and 55·5% and 87·0% were verified, respectively. Non-reciprocal recombination was observed in *dp-b* but not in *b-cn* region with 0·75% ethyl methanesulphonate as evidenced by high frequency of +*b cn* male recombinants over its complementary class *dp*+ +. Majority of the +*b cn* male recombinants verified did not produce in their test cross progeny the two phenotypes (+*b cn* and *dp b cn*) in expected 1:1 ratio contrary to +*b cn* female recombinants, suggesting influence of cytoplasm in transmission of recombinant (+*b cn*) phenotype from male.

Keywords. Induced recombination; genotypic verification; males; *Drosophila melanogaster*.

1. Introduction

Normally, the two complementary recombinant products resulting from a crossing-over event are expected to appear in a 1:1 ratio; non-reciprocal recombination is said to occur when these products fail to appear in this fashion (Strickberger 1979). Non-reciprocal recombination has been found in *Aspergillus*, yeast *Ustilago*, *Drosophila* and *Escherichia coli* (Mohindra 1984). Miglani and Thapar (1983a) observed non-reciprocal recombination with ethyl methanesulphonate (EMS) in males of *D. melanogaster*; the genotypes of the induced recombinants were, however, not verified. The present investigation was, therefore, undertaken on *D. melanogaster* to verify the genotypes of induced recombinants and to study their performance in the next generation.

2. Materials and methods

Two stocks of *D. melanogaster*-a standard 'wild-type' laboratory strain (Oregon-K) and a 'mutant' stock homozygous for 3 second chromosome recessive markers, *dumpy* (*dp*: 2–13·0), *black* (*b*: 2–48·5) and *cinnabar* (*cn*: 2–57·5)–were used. Gene symbols and the map distances are as given by Lindsley and Grell (1968). EMS (Sigma Chemical Co., Batch No. 89c-0439) was used as a probe. For EMS, 0·75% concentration was determined as LD_{50} for the second one-third part of 96 h larval life of *D. melanogaster*, at 25 ± 1°C (Miglani and Thapar 1983b) and was found to be most efficient in inducing recombination in males of *D. melanogaster* (Miglani and Thapar 1983c). It was therefore decided to treat the developing F_1 (Or-K +/*dp b cn*) individuals with 0·75% EMS, mixed with food in ratio 1:9, in the second one-third part of larval life (i.e., 54–86 h after egg deposition) following the method of Miglani and Thapar (1983c). A two-day old F_1 male was crossed with 3 *dp b cn* females to get first testcross (TC_1) progeny. Each of the TC_1 recombinant was again test-crossed to obtain second test cross (TC_2) progeny for verifying its genotype. A TC_1 recombinant was considered as verified if it produced recombinant type flies in addition to *dp b cn* type in TC_2 progeny. All the experiments were done at 25 ± 1°C.

In order to test the difference in the proportion of recombinants in the progeny of control and 0·75% EMS experiments and to determine whether a particular progeny fits into a particular ratio, *z*-test and χ-square tests were used respectively (Gupta 1980).

3. Results

3.1 *Induction of recombination in males*

Out of 14 and 39 F_1 *D. melanogaster* males randomly selected from control and 0·75% EMS experiments, 5 (35·7%) and 32 (82·05%) individuals, respectively, produced a total of 9 and 236 recombinants (table 1). In all these experiments the most prevalent male recombinant phenotype was + *b cn*. Non-reciprocal recombination was thus observed only in *dp-b* region. The number of + *b cn* male recombinants was strikingly higher than that of + *b cn* female recombinants.

Out of 3475 flies produced by control F_1 males, 0·178% were + *b cn* and 0·089%, *dp* + + type recombinants. The 0·75% EMS-treated F_1 males yielded TC_1 progeny

Table 1. Number of second chromosome recombinants recovered (R) in first test cross progeny of *D. melanogaster*, number of recombinants further test crossed (T) and verified (V).

	Control	0·75% EMS
F_1 males test crossed	14	39
Pooled test cross population size	3475	13887
F_1 males yielding recombinants	5	32
Recombinants recovered	9	236
Percent recombinants	0·267	1·699
Male recombinants	R–T–V	R–T–V
+ *b cn*	5–5–1	143–130–106
dp + +	3–3–3	20–14–14
+ + *cn*	0–0–0	4–2–2
dp b +	0–0–0	12–12–12
+ *b* +	0–0–0	1–1–1
	8–8–4	180–159–135
Female recombinants		
+ *b cn*	1–1–1	16–12–11
dp + +	0–0–0	16–12–12
+ + *cn*	0–0–0	6–2–2
dp b +	0–0–0	8–7–7
Total	1–1–1	46–33–32

of 13887, out of which 1·145% were *+b cn*; 0·331%, *dp+ +*, 0·072%, *+ + cn*; 0·144%, *dp b +* and 0·007%, *+b +* type recombinants. Overall per cent recombinants (males plus females) in TC_1 progeny of control (0·267%) and 0·75% EMS experiments (1·699%) were significantly different from each other ($P<0{\cdot}001$).

3.2 *Genotypic verification of TC_1 recombinants*

Number of TC_1 male and female recombinants further test-crossed and verified in TC_2 generation is also given in table 1. The percentages of TC_1 male recombinants verified in control (50%) and 0·75% EMS (84·91%) experiments differed significantly ($P<0{\cdot}01$) from one another. The only class of male recombinants which were not verified was *+b cn*. The only one *+b cn* female recombinant observed in pooled TC_1 progeny of control F_1 males was further test crossed and was verified (table 1). In control and 0·75% EMS experiments combined, irrespective of the phenotype, almost all the TC_1 female recombinants further test crossed were verified.

3.3 *Frequency of recombinant type flies in TC_2 progenies*

In 0·75% EMS experiment, out of a total of 159 male recombinants further test crossed, 24 (all having the phenotype *+b cn*) did not produce any recombinant type flies in TC_2 progeny; only *dp b cn* flies appeared. Such *+b cn* recombinants were considered as unverified. The TC_2 progeny of only the verified recombinants were further analysed. The per cent of flies showing TC_1 recombinant phenotype in TC_2 progeny of a *+b cn* male recombinant from control experiment was 12·2. This figure varied from 0·0–63·6 in 0·75% EMS experiments. An overwhelming majority (100% in control, and 78·3% in 0·75% EMS experiments) of TC_1 *+b cn* female recombinants verified produced the recombinant and *dp b cn* type flies in equal frequency in TC_2 generation (table 2). Thus, in reciprocal crosses (*dp b cn* × *+b cn* recombinant and *+b cn* recombinant × *dp b cn*) differences were conspicuous with regard to the proportion of recombinant type flies in TC_2 progenies.

The *dp b cn* and recombinant type flies appearing in TC_2 progeny of TC_1 recombinants were individually tested for 1:1 ratio (table 2). Combining all the recombinants in control and 0·75% EMS experiments, out of a total of 107 *+b cn* male recombinants verified, TC_2 progeny of only 10 (9·3%) fitted into 1:1 ratio, while out of a total of 12 *+b cn* female recombinants verified, TC_2 progeny of as many as 10 (83·3%) fitted into 1:1 ratio. Quite a high frequency of male and female recombinants of other classes, verified, revealed in TC_2 progeny, the parental and recombinant types in 1:1 ratio (table 2). Thus majority of the *+b cn* male recombinants failed to produce the parental and recombinant type flies in TC_2 progeny in 1:1 ratio.

4. Discussion

4.1 *Spontaneous recombination*

In the present investigation, the control *D. melanogaster* F_1 (Oregon-K*+*/*dp b cn*) males yielded 0·133% verified recombinants in *dp-b* region, at 25°C. Spontaneous

Table 2. Testing the TC_2 progenies of verified *D. melanogaster* TC_1 male recombinants for 1:1 ratio.

TC_1 phenotype	No. of TC_1 recombinants verified	No. of TC_2 progenies showing recombinants		
		= *dp b cn*	< *dp b cn*	> *dp b cn*
Control				
Males				
+ *b cn*	1	0	1	0
dp + +	3	2	1	0
Females				
+ *b cn*	1	1	0	0
0·75% EMS				
Males				
+ *b cn*	106	10	93	3
dp + +	14	12	2	0
+ + *cn*	2	1	0	1
dp b +	12	10	1	1
+ *b* +	1	1	0	0
Females				
+ *b cn*	11	10	1	0
dp + +	12	10	0	2
+ + *cn*	2	2	0	0
dp b +	7	6	0	1

induction of crossing-over was reported in *D. melanogaster* wild-type laboratory strains (Whittinghill and Lewis 1961; Hannah-Alva 1968; Hiraizumi 1973). While studying the effect of temperature on recombination in *D. melanogaster*, no recombinant was observed in test cross progeny of F_1 (Oregon-K + /*dp b cn*) males, at 25°C (Miglani and Thapar 1982).

4.2 *Chemically-induced recombination*

When 0·75% EMS was fed, in the present experiments, to the developing F_1 males in their second one-third part of larval life, 32 out of 39 flies (82·05%) produced recombinants in their progenies. Formaldehyde (0·25% concentration) induced male recombination in third chromosome of *D. melanogaster* in 10% of males tested (Whittinghill and Lewis 1961) while 70·59% of 17 x-irradiated (3,000 r) curly males produced recombinants in their progeny (Whittinghill 1947). The above comparisons revealed 0·75% EMS when given in the second part of larval life to be more effective than formaldehyde and x-rays, at the doses tested, in producing recombinants in higher frequency of treated *D. melanogaster* males.

The overall frequency of recombinants (1·699%) detected with 0·75% EMS, in the present investigation, in *dp-b-cn* region is less than that (3·355%) observed earlier under similar conditions (Thapar 1982). The 0·75% EMS, when given in second part of larval life produced higher frequency of male recombination in *dp-b-cn* region than that (0·189%) with 0·3% EMS given intra-abdominally to adult males (Sharma and Swaminathan 1965).

The present results also indicate superiority of 0·75% EMS (1·699%) over formaldehyde (0·076%) (Sobels *et al* 1959), bleomycin (0·26%) (Demopolous *et al* 1980) and x-rays (0·559%) (Zimmering *et al* 1966) at the doses tested, in inducing male recombination in *dp-b-cn* region of *D. melanogaster.*

4.3 *Reciprocal versus non-reciprocal recombination*

Failure of complementary classes to appear statistically in 1:1 ratio in test cross progeny was interpreted as non-reciprocal recombination (Strickberger 1979). Non-reciprocal recombination was observed with 0·75% EMS in *dp-b* but not in *b-cn* region of *D. melanogaster*, in the present study (table 1). Spontaneous non-reciprocal recombination was observed by various workers using various second and third chromosome genetic markers (Kidwell and Kidwell 1975; Woodruff and Thompson 1977). In our preliminary experiments also, we observed non-reciprocal recombination with 0·75% EMS in *dp-b* but not in *b-cn* region (Miglani and Thapar 1983a). Sharma and Swaminathan (1965) reported non-reciprocal recombination with 0·3% EMS in *dp-b* region. An equal recovery of complementary classes was observed with x-rays in region *b-cn* (Vander Wielen 1979).

With x-rays, non-reciprocal recombination was observed using various second and third chromosome markers (Hannah-Alva 1968; Schacht 1958; Puro 1964). With radio-frequency (Mickey 1963) and neutrons (Kale 1980) also, certain cases of non-reciprocal recombination were reported. Incidence of both non-reciprocal and reciprocal recombinations were observed in various second and third chromosome markers when unfractionated genomic DNA from M-strain or P-strain was injected into M-strain egg (Cronmiller and Cline 1983). Bleomycin induced non-reciprocal recombination in both *dp-b* and *b-cn* regions (Demopolous *et al* 1980). With formaldehyde, non-reciprocal recombination was observed in *Gl-Sb* (Lewis 1957) and *b-pr-vg* regions (Sobels and Van Steenis 1957). Thus various chemicals appear to behave differently in inducing reciprocal/non-reciprocal recombination in various chromosomal regions of *D. melanogaster.*

Non-reciprocal recombination in males of *D. melanogaster* may arise due to induction of recombination, not by classical crossing-over, as in females, but by chromosome breakage and reunion events, as suggested by Woodruff and Thompson (1977). Alternatively, induction of recombination by classical crossing-over, as in females, but preferential elimination of one of the two complementary products may result in what we here observed as non-reciprocal recombination (Sharma and Swaminathan 1965).

4.4 *Confirmation of recombinant genotypes*

In control and 0·75% EMS experiments, respectively, 55·5% and 87·0% of TC_1 recombinants were verified in the present study, as they produced recombinant type flies, in addition to *dp b cn*, in TC_2 progeny. Schacht (1958) further bred 18 x-ray-induced cross-overs involving second chromosome markers in *D. melanogaster* and all of them were verified when bred again. In the present experiments, all recombinants further test crossed were however, not verified. The only class of recombinants some members of which were not verified was + *b cn*. About those + *b* *cn* male recombinants that were not verified, it may be suggested that these individuals may not genotypically be $+b$ *cn*, but they might have looked like $+b$ *cn* flies because of certain developmental modifications.

4.5 *Performance of* TC_1 *recombinants in* TC_2 *progeny*

More than 90% of the $TC_1 + b$ *cn* male recombinants, verified in TC_2 generation, did not produce the recombinant ($+b$ *cn*) and the triple homozygous recessive (*dp b cn*) flies in 1:1 ratio (table 2). In fact, in 78·3% of $TC_1 + b$ *cn* male recombinants verified in EMS experiments, recovery of recombinant type flies was below 15% in TC_2 generation. But on the other hand almost all the $TC_1 + b$ *cn* female recombinants verified produced the two phenotypes in equal frequency (table 2). Thus, in these reciprocal crosses differences were noted with regard to the proportion of recombinant type flies in TC_2 progeny. This suggests that cytoplasm may be playing some role in transmission of recombinant ($+b$ *cn*) phenotype from male. Cytoplasmic influence has been suggested by Luning (1981) with reciprocal crosses over the level of recombination.

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The authors are grateful to Dr Kulbir S Gill, for valuable suggestions during the course of this investigation.

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A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae)

MAHAVIR GUPTA
Laboratory of Animal Behaviour and Simulated Ecology, Department of Zoology, Haryana Agricultural University, Hisar 125 004, India

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Abstract. The sensory receptors of the flagellum of *Apis florea* were studied with simple and transmission electron microscopy. The antenna consists of scape, pedicel and 10 segmented flagellum. Scape and pedicel have only feather like hairs and no structures like the setae or sensillae of the flagellum. The first two segments of the flagellum were covered with setae. The distribution and population of sensilla trichodea, sensilla placodea and sensilla basiconica were determined. Sensilla trichodea were found in maximum numbers and were present on all the flagellar segments. Sensilla placodea and sensilla basiconica were present on 8 distal segments. Transmission electron microscopy studies revealed that the surface of the shaft of sensilla trichodea is serially striated having small permeable points probably indicating pores. The lumen of the sensillum is filled with many dendrites and the dendrites are enclosed in the dendritic sheath.

Keywords. *Apis florea* F.; light microscopy; transmission electron microscopy; sensilla placodea; sensilla basiconica; sensilla trichodea; trichogen cell; tormogen cell; dendrite.

1. Introduction

In the course of evolution, insects have developed various mechanisms to perceive the chemical stimuli and the various types of sensory cells located on the glossa, labial palps, galea and tarsi have been shown to play an important role in perceiving sensory stimuli (Whitehead and Larsen 1976).

Behavioural studies have shown that the bees can perceive chemical stimuli with the antennae present on the head (Dietz and Humphreys 1971; Whitehead and Larsen 1976; Argen 1977). The various types of sensory structures located on the antennae of honeybees have been studied and experimented upon by various workers with light (Vogel 1923) and electron microscopy (Richards 1952; Krause 1960; Slifer and Sekhon 1961; Lacher 1964; Slifer 1967; Kessel and Shih 1974; Callahan 1975).

Depending upon the type of stimulus perceived, the sensillae have been classified into various types (Hodgson 1968; Kafka 1970; Kaissling 1971; Vareschi 1971; Zacharuck 1980). The present studies on the flagellar sensillae of *Apis florea* were undertaken in order to understand their ultrastructure and the type of sensillae present on each segment and their distribution.

2. Materials and methods

Freshly emerged worker bees were obtained from the colonies maintained in apiaries. Immediately after capture the antennae were clipped and immersed in 2% KOH (4–6 h) at 60°C for decolouration and preparation of temporary mounts in glycerine were prepared to study the types and distribution of sensillae.

2.1 *Light microscopy*

The tissue for light microscopy was processed by usual methods. The decolourised antennae were embedded in paraffin and serial sections were out at 10 μm and stained in methylene blue.

2.2 *Electron microscopy*

The antennae of freshly emerged workers were obtained and processed following the method of Harbach and Larsen (1976).

After clipping, the antennae were fixed in 2·0% glutaraldehyde (pH 7·4) and placed under vacuum for 2–3 h. The material was washed in phosphate buffer (pH 7·4) and post fixed in 1·0% osmium tetraoxide for 2 h and again washed in phosphate buffer (pH 7·4), dehydrated in ethanolic series and embedded in araldite mixture in gelatin capsules (Luft 1961). The ultrathin sections were cut with LKB-8800 ultratome at 700 Å and double stained in uranyl acetate and lead citrate for 1 h (Reynolds 1963) and examined under Philips EM 300 G microscope.

3. Results and discussion

The length of the flagellum varies from 3·0–3·5 mm. The proximal two segments are shorter than the rest. The last segment has a blunt roundish ending (figure 1). Scape and pedical have only setae and are completely devoid of sensillae (figure 2). The flagellum of worker bees of *A. florea* has 10 segments.

No significant differences on the population counts of the various types of sensillae between the right and left antenna were observed (table 1).

The segments starting from proximal to distal end of antenna are designated as Se_1 upto Se_{10}. On the basis of morphology described by other workers (Slifer and Sekhon 1961; Argen 1975) 3 types of sensillae were identified from the antenna of *A. florea* (figure 3).

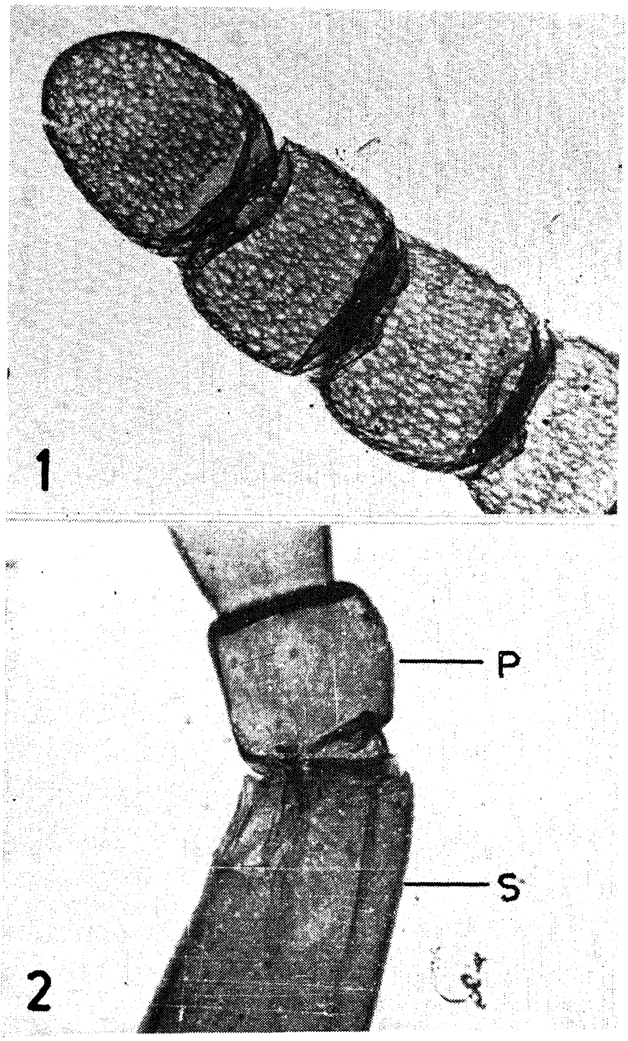
(i) Sensilla placodea having 59–399 μm in area with an average of 197 μm are oval pore plates with slightly thick raised rim. They are distributed from the proximal end of Se_3 to the last segment Se_{10}.

(ii) Sensilla trichodea are slender and straight, 13–22 μm in length with an average of 17 μm and their width is 0·85–2·2 μm with an average of 1·5 μm. They are found on all the segments of the flagellum.

(iii) Sensilla basiconica are 8–19 μm long with an average of 13 μm and 1·7–3·4 μm wide with an average of 2·6 μm. These sensillae have blunt tips.

Setae are needle like and uniformly distributed on all the segments of the flagellum, however, the first two segments of the flagellum are dominated by setae. The setae are 2–33 μm long with an average of 15 μm and 0·68–2·2 μm wide with an average of 1·3 μm. The setae present on the inner side of the antenna were longer.

Most of the sensory structures found on the antennae were situated on the 8 distal segments. The remaining two segments of the flagellum were exclusively covered with the sensilla trichodea. Sensilla placodea and setae occupy distinct zones on the segments (distal 8) with very little overlapping. However, no clear demarcation between the sensillar and setal zone was observed. The surface of antenna is rough in



Figures 1 and 2. **1.** Distal segment of the flagellum of *A. florea* F. Note that the last segment has a blunt roundish ending (× 200). **2.** Basal part of antenna of *A. florea* F. showing Scape (S) and Pedicel (P) (× 200).

the setae rich zone and smooth in sensilla placodea rich zone. A part of the last segment has very few sensillae and appear glossy.

There were not much differences in the distribution of sensilla trichodea on the two sides of the antenna (1726 on upper side and 2034 on under side). However, the population of sensilla placodea and sensilla basiconica is different. The population of former being 3 times higher on the upper side while the later is 7 times more on the under side of the antenna.

The average total population of the sensillae is in the following order: sensilla trichodea > sensilla placodea > sensilla basiconica.

The sensillum is composed of sensory cells, formative cells and accessory cells

Table 1. Distribution of olfactory receptors on the flagellar segments of the antennae of worker bees of *A. florea*. Population counts are an average of 15 antennae of each side.

Segment No.		Sensilla placodea		Sensilla trichodea		Sensilla basiconica		Total
		UP	UN	UP	UN	UP	UN	
X	L	72·4 ± 2·4	28·6 ± 2·1	166·0 ± 3·4	151·1 ± 2·8	19·4 ± 1·4	4·1 ± 0·20	441·6
	R	71·0 ± 2·0	24·1 ± 2·8	164·0 ± 3·9	144·2 ± 4·9	18·0 ± 2·8	3·2 ± 0·2	424·5
IX	L	70·2 ± 1·8	24·3 ± 2·6	136·3 ± 2·4	148·0 ± 4·0	14·1 ± 3·1	2·1 ± 0·1	395·0
	R	69·0 ± 1·2	21·1 ± 2·4	131·5 ± 3·4	141·2 ± 4·1	13·2 ± 2·1	1·0 ± 0·2	377·0
VIII	L	68·0 ± 2·6	24·5 ± 2·9	120·0 ± 4·1	141·3 ± 4·0	14·0 ± 4·0	1·2 ± 0·1	369·0
	R	68·0 ± 2·1	19·0 ± 2·4	114·1 ± 4·0	134·2 ± 4·1	12·5 ± 4·1	1·0 ± 0·1	348·8
VII	L	62·4 ± 1·4	21·4 ± 3·4	99·0 ± 3·9	134·0 ± 3·9	11·1 ± 3·1	1·3 ± 0·2	329·2
	R	60·0 ± 3·1	19·2 ± 3·1	81·1 ± 3·4	119·2 ± 3·8	11·2 ± 2·8	1·3 ± 0·1	292·0
VI	L	60·0 ± 2·4	20·2 ± 2·9	80·0 ± 3·9	114·1 ± 4·0	9·1 ± 3·1	1·0 ± 0·3	284·4
	R	60·2 ± 2·1	19·0 ± 1·9	80·2 ± 3·1	104·1 ± 4·8	9·1 ± 2·8	1·2 ± 0·1	273·8
V	L	60·0 ± 1·8	18·2 ± 2·6	76·0 ± 4·0	101·0 ± 4·1	8·0 ± 2·1	1·0 ± 0·1	264·2
	R	58·0 ± 1·6	16·6 ± 2·9	71·1 ± 3·8	96·2 ± 4·0	4·1 ± 2·0	1·2 ± 0·2	247·2
IV	L	48·7 ± 1·9	14·0 ± 3·1	72·0 ± 4·1	94·0 ± 3·9	3·0 ± 1·8	3·0 ± 0·1	232·7
	R	41·0 ± 2·0	12·3 ± 2·1	71·1 ± 4·6	81·1 ± 4·1	3·2 ± 1·6	0	208·7
III	L	41·0 ± 2·8	12·2 ± 2·0	52·1 ± 4·9	81·0 ± 4·0	1·0 ± 0·2	0	187·3
	R	32·1 ± 3·1	11·0 ± 1·8	54·0 ± 4·0	68·4 ± 2·5	0	0	165·5
II	L	0	0	51·0 ± 3·9	61·0 ± 2·9	0	0	112·0
	R	0	0	41·1 ± 3·4	45·3 ± 3·0	0	0	86·4
I	L	0	0	35·0 ± 3·0	41·0 ± 3·1	0	0	76·0
	R	0	0	31·1 ± 3·9	34·2 ± 3·4	0	0	65·3
Total		942·0	305·7	1726·7	2034·6	151·0	20·6	5180·6
	L	482·7	163·4	887·5	1066·5	79·7	11·7	2691·5
	R	459·3	142·3	839·2	968·1	71·3	8·9	2489·1

UP, Upper side (The flagellum side against the head).
UN, Under side (The flagellum side away from the head).

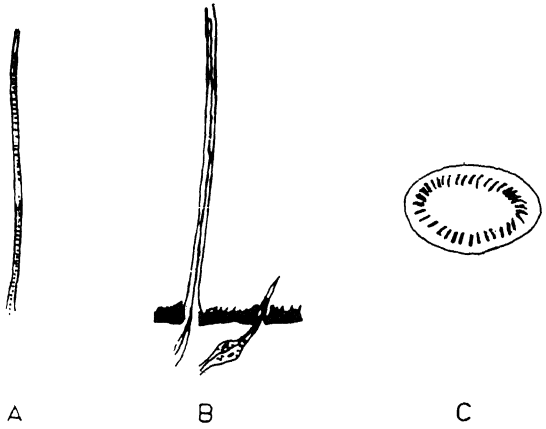


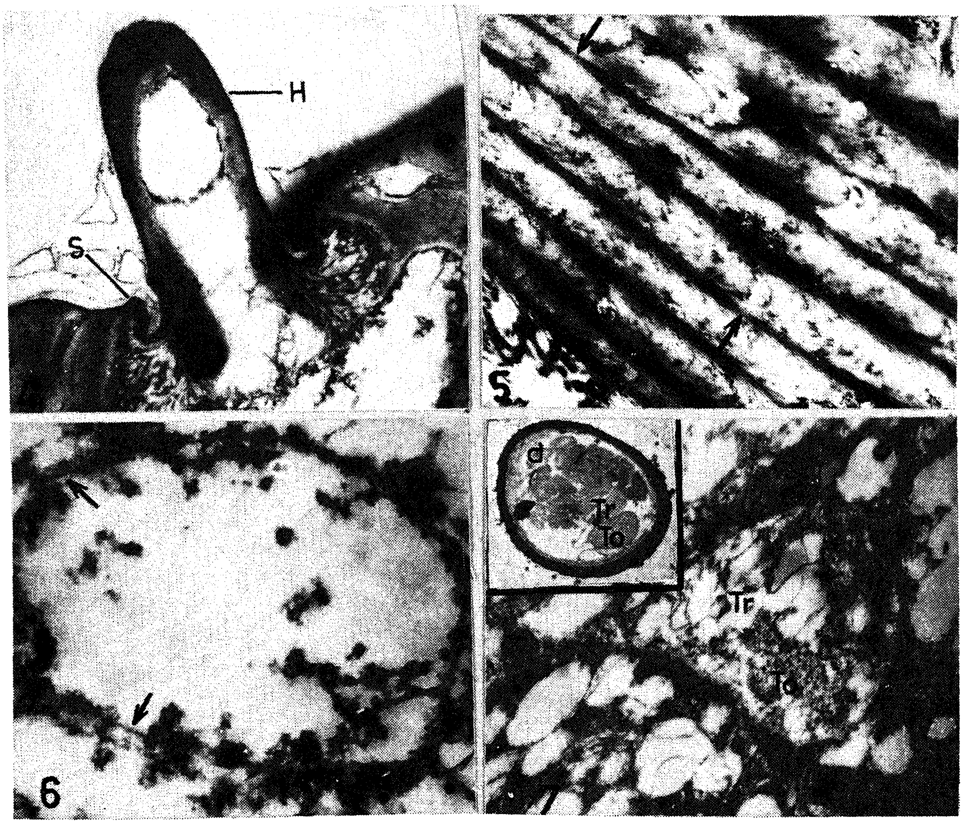
Figure 3. Diagrammatic representation of olfactory sensillae in *A. florea* F. **A.** Sensillum trichodeum. **B.** Sensillum basiconcum. **C.** Sensillum placodeum.

ogether with a cuticular hair (Ernst 1969) (figure 4). The wall of the hair is serially triated with weak permeable points (figures 5, 6), however, true pores in the walls of he sensillum could not be observed. Trichogen and tormogen cells which lie at the ase of the sensillum show enfolded relationship (figure 7). The cuticular sheath at he base of the sensillum encloses many dendrites (figure 8). Perikarya gives rise to endrites that pass extracellularly through the trichogen cell. Prior to entering the xtracellular vacuole of the tormogen cell, dendrites are held tightly in a glial omplex which is bound by septate desmosomes. The wall of the sensillum encloses ensillum liquor and dendritic branches (figure 9). The wall of the dendrite is onsolidated at the tip of the sensillum (figure 10).

In the present studies only 3 types of sensillae on the antenna of *A. florea* were dentified. On the basis of behavioural studies (Gupta 1982) olfactory functions have een assigned to these sensillae. These observations are in accord with those of other orkers (Lacher 1964; Whitehead and Larsen 1976) on *A. mellifera.* With scanning lectron microscope many workers have identified more than 5 types of sensillae resumed to be olfactory in function in other insects (Richards 1952; Slifer and ekhon 1961; Kessel and Shih 1974; Callahan 1975; Argen 1977) including bees like . *mellifera.*

The location of these receptors in *A. florea* are similar to that of *A. mellifera.* owever, the population of these receptors is comparatively much less than . *mellifera.* On the antennae of *A. florea* the number of sensilla trichodea varied rom 1726–2034, sensilla placodea from 305–942 and sensilla basiconica from 20–151 table 1), while in *A. mellifera* the number of sensilla trichodea ranged between 6000 nd 9000, sensilla placodea 3000 and 3500 and sensilla basiconica 100 and 150 (Dietz *t al* 1974).

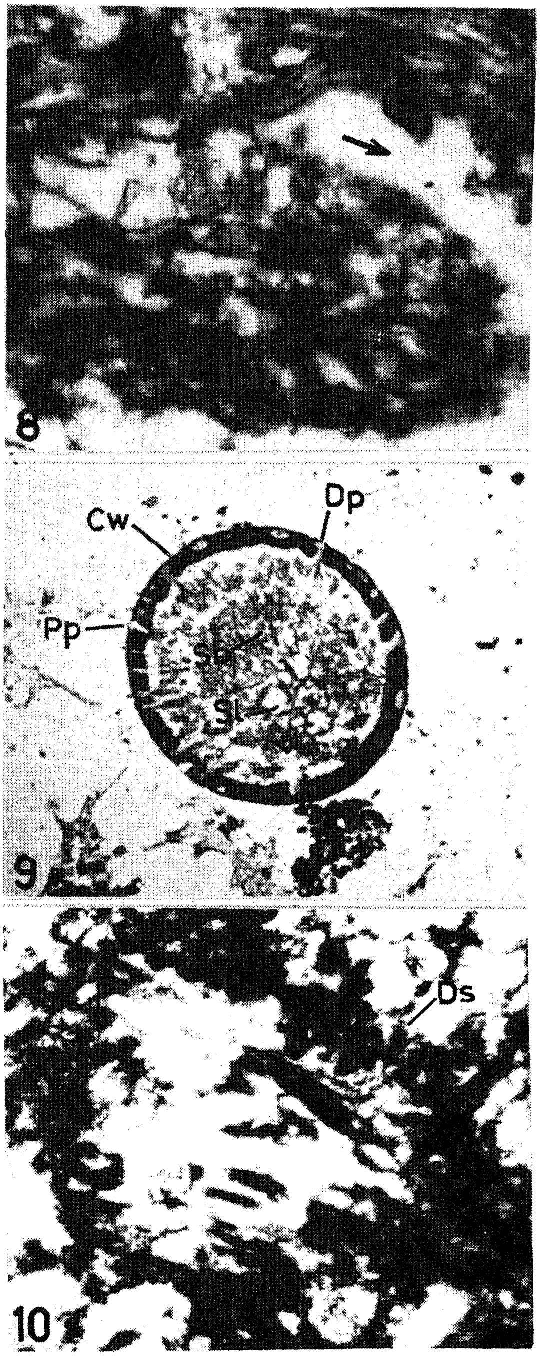
The olfactory stimuli have an extraordinary significance for mutual ommunication among the bees and the dances exhibited by them are in fact the xpression of communication and olfactory perception. *A. florea* show a simple orizontal dances under semi-open sky and is considered to be of a primitive type nd is an initial step towards the dance performed on a vertical plane by other bee



Figures 4–7. **4.** Electron micrograph of a cross section through distal antennal segment showing the base of a sensillum. H, Hair; S, socket (× 14520). **5.** Electron micrograph showing tangential section of a shaft of a sensillum trichodea showing serial striations of the wall and penetration of weak permeable points (arrows) (× 23760). **6.** Electron micrograph of a cross section through a sensillum trichodea showing weak permeable points (arrows) in cuticular wall (Cw) (× 23760). **7.** Electron micrograph of a cross section of a sensillum trichodea epidermal cell complex showing enfolded relationship between trichogen (Tr) and tormogen (To) cell (× 11880). *Inset.* Transverse section through a sensillum trichodea. Note the trichogen (Tr) and tormogen (To) cells (× 100).

species (Lindauer 1956). Therefore it is assumed that the sense of olfaction in *A. florea* is comparatively poorly developed than in other bee species since the population of the olfactory receptors on the antennae are comparatively lower.

The permeable points observed in sensilla trichodea in the present studies are similar to those of sensilla basiconica and sensilla placodea (Slifer and Sekhon 1961). The pores in sensilla placodea are arranged within a ring shaped pore field and between the rows of pores radial ridges, connecting surrounding cuticle with the central plate are also present. The pore system in the walls of olfactory sensillum are the routes through which the odour molecules enter the olfactory hair (figure 11). In *Bombyx mori*, not only the pores but also the total surface serves as a target for odour molecule (Steinbrecht 1969). Therefore, the molecules adsorbed between the pores are able to reach inside the sensillae by diffusion, probably through the outer lipid layer of the cuticle (Adam and Delbruck 1968) or through the pore tubules.



Figures 8–10. **8.** Electron micrograph of a longitudinal section through the base of a sensillum. Note cuticular sheath encloses many dendrites and one dendrite (d) is seen leaving (arrow) through the opening (× 23760). **9.** Vertical section through a sensillum trichodea. Note the sensillum with many permeable points. Many dendritic and subdividing branches surrounded by sensillum fluid can also be seen. Cw, Cuticular wall; Dp dendritic protrusion; Pp, permeable points; Sb, subdividing branch; Sl, sensillum liquor (× 500). **10.** Electron micrograph showing cross section of a dendrite from the tip of a sensillum. Note dendritic sheath (Ds) is more consolidated in this area (× 23760).

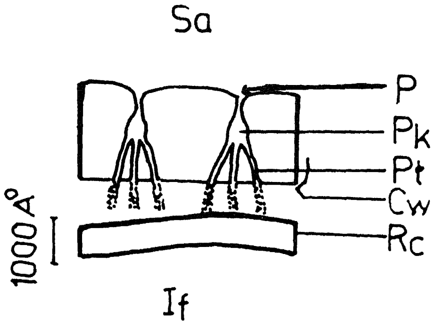


Figure 11. Schematic diagram showing contact between tubules and dendrites. Cw, Cuticular wall; If, internal fluid; P, pore; Pk, pore kettle; Pt, pore tubule; Rc, receptor cell dendrite; Sa, surrounding air.

SEM and SEM cracking method can probably be other techniques to obtain additional morphological information. However, specific odours to which given receptors are sensitive may be further determined by other techniques such as electrophysiological investigations.

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Ontogeny of play behavior in free-ranging rhesus macaques (*Macaca mulatta*)

RAJBIR SINGH*
Department of Psychology, University of Rajasthan, Jaipur 302 004, India
*Present address: Department of Psychology, Kurukshetra University, Kurukshetra 132 119, India

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Abstract. Self and social play of 29 rhesus monkeys were observed in a free-ranging group situation for 6 months. Subjects of various age-sex classes were observed by focal animal and one-zero sampling. Frequencies for 5 categories of play were analyzed in a 3 way factorial analysis of variance. Age and frequencies of play were found to be inversely related. Analysis also revealed that except during infancy, females played more than male subjects. Among categories, self play and non-contact mutual play were dominant forms than those involving physical contact of either or both partners. However, it was also influenced by age, as the preference shifts from self play to non-contact mutual play in a developmental perspective. Among adult monkeys, the occurrence of play was least. Obtained results have been interpreted in terms of ontogenetic and situational variables. Rhesus monkeys preferred peers in social play than youngers and elders. However, adolescent/subadult and adult animals played with younger animals.

Keywords. Ontogeny; self-play; social play; free-ranging rhesus monkey.

1. Introduction

Play behavior among mammals has been identified as an activity with substantial evolutionary, biological and ethological significance (Wiesler and McCall 1976; Vandenberg 1978; Fagen 1981). Research on primate play has advanced many functional propositions relating to dominance, group and role behaviors (Loizos 1967; Poirier and Smith 1974). Symons (1978) has presented a detailed dynamics of the development of aggressive behavior through play among rhesus monkeys. In experimental situations, play has been found indispensable for adequate social development in rhesus monkeys (Mitchell 1970; Suomi 1974). In nature, rhesus monkeys start play in early weeks of life and continue even in adulthood with less frequencies (Southwick *et al* 1965; Lindburg 1971; Bhan *et al* 1984). Mitchell (1979) has reviewed sex-differences in rhesus play patterns.

Despite the proliferation of studies on rhesus play, a detailed ontogenetic description of play behavior from naturalistic settings is really lacking. Therefore, the present study was planned to account for the occurrence of self and social play in a free-ranging rhesus group. It attempts to describe the play behavior of subjects varying in sex and age from birth to old age.

2. Methods

2.1 *The study area and group*

A population of about 350 rhesus monkeys inhabits in Galta hillock, eastward to Jaipur (Rajasthan). It is distributed in 4 groups of varying sizes with frequent intergroup interactions. Among the 4 groups, two groups were bigger than 100 and often intermixed, which made observations very difficult. The third group was smaller than 40 and could not provide the sample as required. Therefore, the fourth group of moderate size and big home range was selected. The size of the study group in the last week of October 1980 was 73. It ranges in an area of about 4 km^2. Being an arid rocky zone, there are few big trees in the area and for their safety the group used the same as sleeping site during the study period. The site is also important for devotees of Hindu religion and, therefore, the group is abundantly offered food with varied human interaction.

2.2 *Subjects*

A total of 29 rhesus monkeys served as subjects. The subjects were selected on the basis of age, sex, spatial position in the group, identification marks and kinship group. For example, when juvenile 1 (J_1) was selected its sibling was not selected from juvenile 2 (J_2) category or infant category. Such criteria ensured full representation of group interactions. Table 1 describes their particulars. As the group was under observation since 1977 only, age estimations for mature animals and those born earlier were approximate on the basis of physical/behavioral characteristics and birth seasonality. Therefore, adults were categorized as young, mature and old. Nulliparous, primiparous and diparous females were categorized as young; multiparous as mature, and non-reproductive with twisted tail and broken teeth as old. Among males, peripheral, low ranked with smaller but red testes as young; heavy with developed canines and testes as mature; and with twisted tail and rubbed extermities as old. One juvenile female (JF_1) died in August 1980 because of some

Table 1. Particulars of subjects (N = 29).

Class	Sex	Age (May–October, 1980) (Months)	Codes	n
Infant	Female	Birth-5	I_1, I_3	2
	Male	Birth-6	I_2, I_4	2
Juvenile$_1$	Female	13–18	FI_1, FI_2	2
	Male	13–18	MI_1, MI_2	2
Juvenile$_2$	Female	25–30	JF_1, JF_2	2
	Male	25–30	J_1, J_2	2
Adolescent	Female	37–42	AF_1, AF_2	2
	Male	37–42	J_3, J_4	2
Adult	Female	Young, 49–54	YF	
		Multiparous Mature	MF_1, MF_2	5
		Non-reproductive old	OF_1, OF_2	
	Male	Young transitional 49–54	MJ_1, MJ_2	
		Young peripheral 61–66	SA_1, SA_2	
		Young central	YM	8
		Mature-full sized	MM_1, MM_2	
		Old	OM	

unspecified disease. One young male (SA_1) left the group in August 1980 and joined another group in the Jaipur city. One old female (OF_1) died in the first week of July 1980 as it slipped from a high cliff. Female infants were born during May 1980, i.e., after the beginning of actual observations (I_1–31st May; I_3–13th May)

3. Procedure

3.1 *Observation schedule*

Observations were taken by focal animal and one-zero sampling technique (Altmann 1974) on a checklist with 20 s intervals. Each animal was observed for 10 min (30 intervals) twice a week for 6 months, viz from Ist week of May to last week of October, 1980. Thus, actual observations covered a duration of 13,920 min (session × week × months × N subjects). However, 48 sessions could not be materialized due to deaths of two subjects and migration of one male during the study.

3.2 *Definitions of play categories*

On going behaviors were recorded by codes on checklist with specification of the interacting animal(s) with the focal animal. Sex of infants and J_1 during interactions was not specified for the low reliability. The social interactions involving more than 4 animals were not analyzed and recorded as subgroup activity. Play behaviors analyzed in the present study were classified into 5 broad categories and defined as following:

(i) Self play—any rolling, repetitive climbing, jumping, running and playing with inanimate object and animate other than conspecifics.

(ii) Non-contact mutual play—visually oriented charges approaches, and withdrawals with another animal(s).

(iii) Mutual contact play—rough and tumble nature, wrestling and shambiting etc.

(iv) Non-mutual contact play with others—any rough handling, play instigation by focal animal while the interacting animal remained passive.

(v) Non-mutual contact play by others—elements as in (iv) by interacting animal, while the focal animal remained passive.

3.3 *Scoring and analysis*

Frequencies of occurrence for 5 categories were summed up for each age-sex class. Among adults, the occurrence was so low that scores of sub classes, viz young, mature, and old had to be mixed. Since, the number of animals in age-sex classes was unequal, for the equal limits mean scores were obtained. Thus, the maximum possible occurrence for self play was 1440 (48 sessions × 30 intervals per session). For the categories of social play, focal animal could interact with 7 age sex classes and upto 3 animals from each class. Thus, theoretically the maximum possible occurrence for social play categories could be 31240 (21 social interactions × 48 sessions × 30 intervals/session).

The observed frequencies were arranged in a 2 × 5 × 5 trivariate table, viz male-female, 5 age classes (infant, J_1, J_2, adolescence/subadult and adult); and the 5 play categories. It was analyzed for variance by Tukey's non-additivity test (Winer 1971). The F value was considered significant having associated probability equal to or less than 0·01. The second order interaction served as error term because non additive F was non significant and residual could not be error term.

In another analysis, only the social play was analyzed to see the effect of the age of the partner at 3 levels, viz youngers, peers and elders. Thus, 2 × 5 × 3 factorial analysis of variance with Tukey's test was done. It involved 3 variables, as the sex, age class and the age of interacting animal.

4. Results

The sex of animal as an independent source of variance was not found to be significant. Mean play frequencies for females (26·53) were nonsignificantly higher than for males (23·68).

Significant differences emerged among age classes (table 2). Play behavior frequencies decreased with increasing age. Infants was the most playful class, whereas the adults played with the least frequencies (figure 1). Various play categories were emitted with significantly different frequencies ($F = 19{\cdot}50$, df. = 4/16, $P < 0{\cdot}001$). Non-contact mutual play was the most preferred category with its mean occurrence as 38·52, followed by self play with mean as 35·98 (figure 2).

An interaction between age and sex was found to be significant ($F = 15{\cdot}83$, df. 4/16, $P < 0{\cdot}001$). Figure 3 shows that males play more than females during infancy, but is vice versa in other age classes (table 3). Sex differences in relation to categories of play was non-significant, although, a significant interaction effect between age class and categories of play was obtained ($F = 5{\cdot}04$, df. 16/16, $P < 0{\cdot}01$). Infants and younger juveniles (J_1) exhibited the highest frequencies for self play, whereas elder juveniles (J_2) and subadults/adolescents revealed highest frequencies for non-contact mutual play (table 3).

The analysis of social play (table 4) revealed that the age of focal and interacting animals had a significant effect on play frequencies ($F = 6{\cdot}10$, df. 8/8, $P < 0{\cdot}01$). Immature animals preferred to play with peers than with youngers or elders, whereas subadult/adolescent and adult animals played more with younger animals than with peers (figure 4).

Table 2. Mean frequencies of play at different levels of main sources.

Source / Levels	Sex F = 1·41(1/16)ns		Age-class F = 58·94(4/16)	$P < {\cdot}001$	Type of play F = 19·50(4/16) $P < {\cdot}001$	
1	Male	23·68	Infant	53·60	Self play	35·98
2	Female	26·53	Juvenile$_1$	37·95	Contact play with others	22·63
3			Juvenile$_1$	17·50	Contact play by others	11·37
4			Subadult/ Adolescence	14·70	Non-contact mutual play.	38·52
5			Adult	1·76	Mutual contact play	17·02

ns, Non-significant.

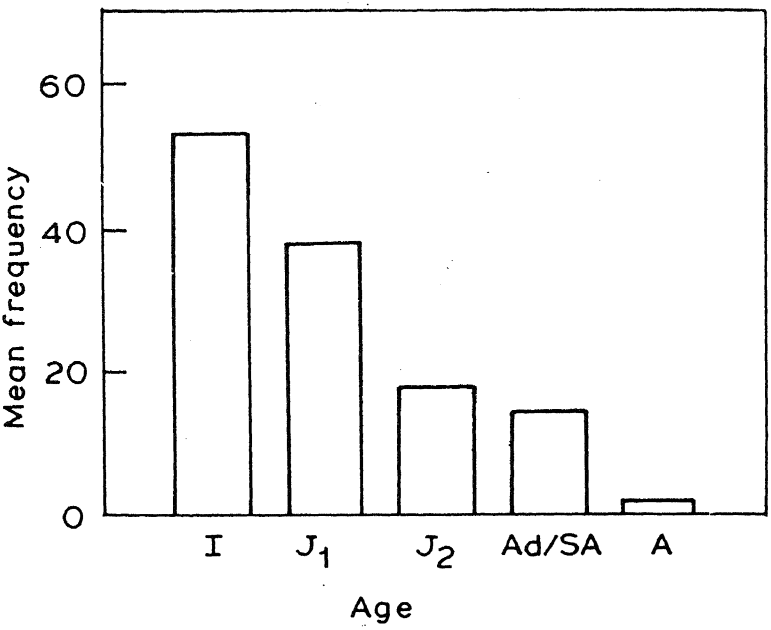

Figure 1. Mean frequency of play at different age levels. I, Infant; J_1, juvenile 1; J_2, juvenile 2; Ad/SA, adolescent/subadult; A, adult.

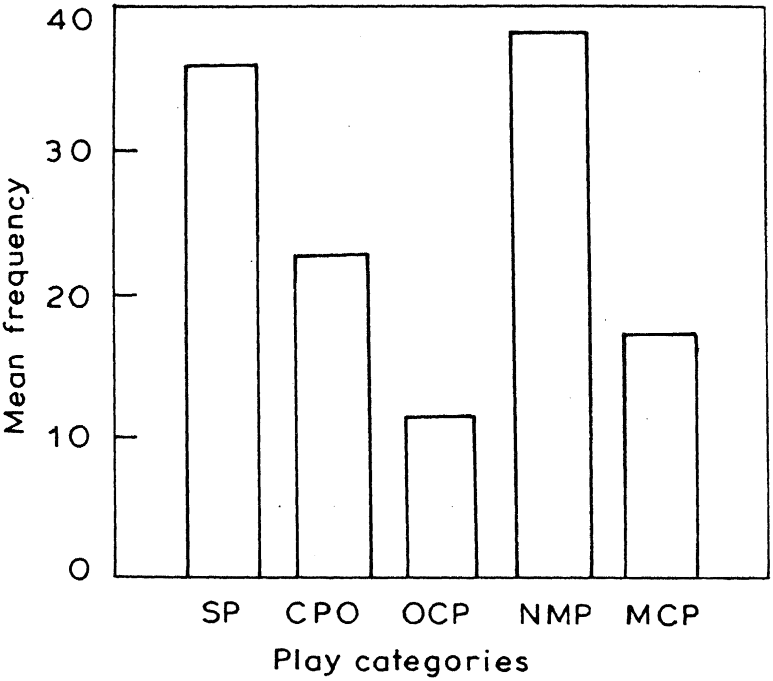

Figure 2. Mean frequencies of different categories of play. SP, self play; CPO, contact play with others; OCP, others contact play; NMP, non mutual contact play; MCP, mutual contact play.

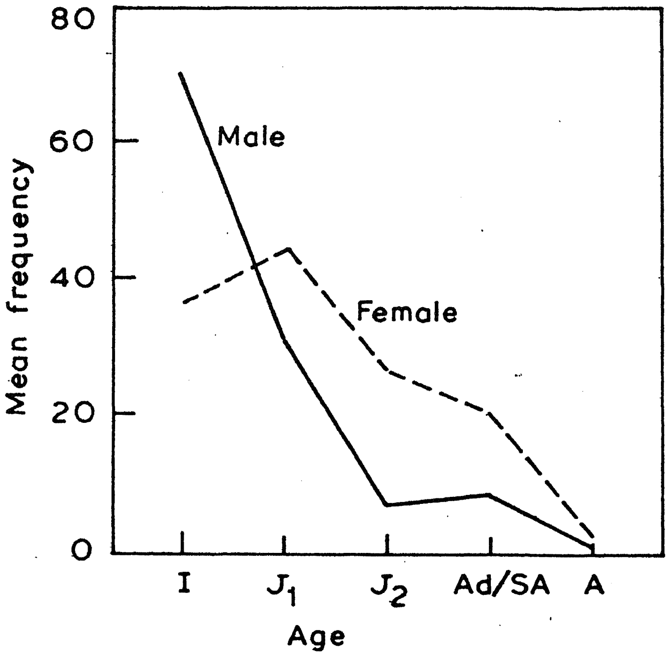

Figure 3. Mean play frequencies of both sexes at various age levels. I, Infant; J_1, juvenile 1; J_2, juvenile 2; Ad/SA, adolescent/subadult; A, adult.

Table 3. Significant interactive means of play frequencies.

	Infant	Juvenile$_1$	Juvenile$_2$	Subadult/ Adolescent	Adult
Sex × age-class					
Males	70·20	31·30	7·10	8·80	2·00
Females	37·00	44·60	27·90	20·60	2·53
Type of play × age-class					
Self play	98·25	53·00	17·00	11·25	0·39
Contact play with others	49·50	37·00	14·50	14·50	3·65
Contact play by others	22·50	19·00	5·25	9·25	0·84
Non-contact mutual play	70·00	52·75	38·00	29·25	2·60
Mutual contact play	27·75	34·00	12·75	9·25	1·34

Table 4. Interactive means of social play for age-class × age of interacting animal.

Age of Interacting animal	Youngers	Peers	Elders
Age-Class			
Infant	—*	76·75	45·00
Juvenile$_1$	51·25	57·25	4·00
Juvenile$_2$	10·75	47·75	6·75
Adolescent/subadult	51·00	1·50	0·50
Adult	4·33	0·17	—*

*Interaction not possible, since infant was the youngest and adult was the eldest class.

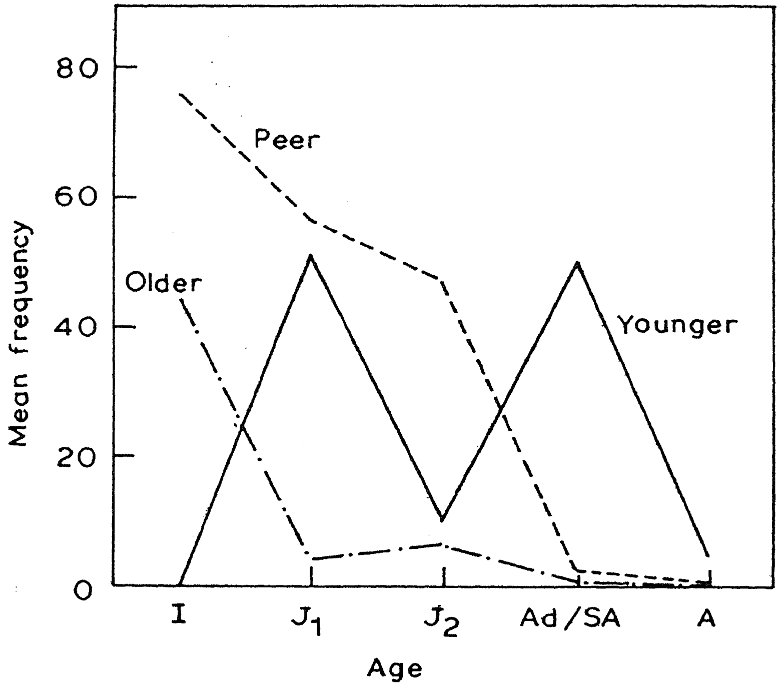

Figure 4. Mean frequencies of social play with age of interacting partner at different age levels. I, Infant; J_1, juvenile 1; J_2, juvenile 2; Ad/SA, adolescent/subadult; A, adult.

5. Discussion

It is generally understood that males among rhesus monkeys are more playful than females. Results of the present study did not conform to this notion. Although sex differences exist, but these concomitantly change with age. It is only during infancy that males play more than females. In rest of the ontogeny females play more than males. This change can be attributed to the shift in the spatial position of growing male in the group space. Juvenile and subadult male monkeys are propelled toward the periphery by group pressures. Consequently, the chances of play interactions are minimised. Whereas juvenile and adolescent female monkeys continue to stay with female assemblies and, thus, interact quite frequently with infants and peers. Moreover, the pattern of play of male and female young monkeys also contribute in age and sex interaction effect. Play bouts of juvenile and subadult males terminate soon, as these become rougher and aggressive. On the other hand, female play bouts last longer and rise to long term play sequences. Thus, sex differences in rhesus play are influenced by factors such as age, social structure, type of play, and spatial position in the group which operates characteristically in free-ranging situations.

Play frequencies decreased linearly with advancing age levels. Such a trend seems to be in congruence with learning theories (Fagen 1981). As by the end of adolescence age, monkeys, by and large, complete the socialization process and explore the major features of home range, less functional aspect of play is left beyond this age. Within adulthood, young adults were observed as playing several times and all forms of play did occur. However, mature and old animals indulged in play rarely, i.e., only in 10 instances. Such occassional play by rhesus adults has also been reported by Lindburg (1971) in free ranging groups.

Different kinds of play patterns were exhibited by animals of different age-classes. During infancy, self play was most prominent and it was generally directed toward environment. It helps the infants in speedy exploration of the environmental features for which these animals are highly motivated (Harlow 1956). Among social play categories, non-contact mutual play was preferred most during other age levels. This is the safest category in the sense that contact play frequently terminate into aggression.

Peer play has received lot of attention in primate play studies and its importance has been evaluated (Harlow 1969). Analysis of the present study revealed that peers were most sought partners in the social play of infants and younger juveniles. Although infants play with elders quite frequently, juveniles do not do so. Elders often harrass youngers in social play, but infants are continuously attended by their mothers and so they are saved by elders' harrassment. Adolescent/Subadult monkeys play more with youngers, because their peer play becomes aggressive and elders seldom play.

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Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes

P H PATHAK and S S KRISHNA*

Entomology Laboratory, Department of Zoology, University of Gorakhpur, Gorakhpur 273 009, India

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Abstract. An extremely low egg output, almost approaching zero level, and with no sign of viability, occur in *Earias vittella* (F.) when the females are coupled for mating with antennectomised males or if both sexes paired for coition are wingless. However, sexual union arranged between normal females and males whose both wings or only fore or hindwings are excised facilitates these insects to deposit fertile eggs whose number, however, always remains markedly lower (more so when the males in the breeding pairs are without hindwings) in comparison to that recorded for mated females in pairs where both sexes are normal.

Keywords. *Earias vittella*; oviposition; egg viability; antennectomy; alactomy.

1. Introduction

Mishra and Krishna (1981) reported that normal females of rice moth, *Corcyra cephalonica* (Stainton), a serious pest of stored commodities (Piltz 1977), when paired with antennectomised or alactomised males, do not lay any viable egg. Taking this as a cue, it was considered desirable to ascertain to what extent the egg output and egg viability would become affected in another, though taxonomically unrelated, moth, *Earias vittella* (F.), a major pest of cotton and okra in the tropics (Mehta 1977; Butani and Jotwani 1984), by antennectomy or removal of wings (one of the two pairs or both) in males involved in mating or alactomy *in toto* of individuals belonging to both sexes coupled for copulation. We present our data obtained from such a study in this communication.

2. Material and methods

For this investigation, adults of either sex reared individually in the laboratory on developing seeds separated from okra fruit (Vishwapremi and Krishna 1974) were used. Antennae or fore and/or hindwings of males or both alae of the two sexes were extirpated completely from their bases with a pair of Dewecker's scissors and the cut ends of these appendages in the body of the moths were immediately dressed with Savlon antiseptic cream to check possible subsequent infection and also to block further bleeding.

Following this operation, the insects were housed individually for 24 h in muslin-covered glass containers (70 mm diameter; 90 mm height), provided with a hanging glass capillary filled with 15% sucrose solution that formed the food of the adults, to enable them to recover from any postoperational shock that might otherwise interfere during experimentation. At the end of 24 h, such an antennectomised or a partially or wholly wingless male was paired for mating with a normal (in which both antennae and wings were intact), newly eclosed individual of the opposite sex inside another glass container, similar in all respects to the one in which moths were kept soon after surgery, that served as the oviposition chamber. A totally wingless male and a female were also coupled for mating and formed an independent experimental set in this study. The monitoring of egg laying during the first 4 days of oviposition (the period when mated females in their life time deposited most of their eggs) was similar to that described earlier (Vishwapremi and Krishna 1974) and egg hatchability in these tests was also recorded. All trials were adequately replicated and were performed at temperatures ranging between 25°C and 28°C and relative humidity varying from 80–100%. The data obtained were compared with those collected from separately arranged control tests which included normal males and females and, wherever desirable, subjected to appropriate statistical analysis (Paterson 1939) for interpretation.

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*To whom correspondence should be addressed.

3. Observations

Very few eggs, all non-viable, were laid by normal females of *E. vittella* coupled with males devoid of antennae or by wingless females closeted with males sans wings (table 1). Partial alactomy (removal of fore or hindwings) or complete loss of wings of males, although did not hamper mating of these individuals with normal females, resulted in such copulations the outcome of which always yielded pronouncedly reduced number of total and viable eggs, more so when the males in the breeding pairs were without hindwings (table 2).

4. Discussion

Loss of both antennae in males of *E. vittella* appears to have fully incapacitated these individuals to perceive the female sex pheromone reported to be present in this

Table 1. Estimates of oviposition and hatchability of eggs in *E. vittella* in tests where both sexes of copulating pairs were normal (control) or without wings or where males in such couples were devoid of antennae (data pooled from 5 females).

Experimental condition	Mean number of total eggs laid	Mean number of viable eggs laid
Normal females paired with normal males (control)	312·2	258·0
Normal females paired with antennectomised males	2·2	0
Alactomised females paired with wingless males	0·6	0

Table 2. Estimates of oviposition and hatchability of eggs in *E. vittella* in tests where both sexes of copulating pairs were normal (control) or where males in such couples were completely wingless or were devoid of fore or hindwings (data pooled from five females)*

Experimental condition	Mean egg output/female	Mean egg viability/female
Normal females paired with normal males (control)	312·2 a	258·0 a
Normal females paired with males devoid of forewings	183·4 b	141·4 b
Normal females paired with wingless males	121·8 b	84·4 b
Normal females paired with males devoid of hindwings	93·8 b	44·4 b
Mean	177·8	132·1
LSD (1%)	141·1	133·8
(5%)	102·4	97·1

*Means in the same vertical column followed by the same alphabet do not differ significantly at the 1% or 5% level by the least significant difference (LSD) test.

noctuid species (Campion 1984) (information based on a private communication received by Campion from B F Nesbitt and R Baker). This, in turn, plausibly, must have miserably handicapped these males in the performance of any of the steps of orientation associated with precopulatory behaviour leading to mating. In this respect, *E. vittella* resembles the rice moth, *C. cephalonica* (Mishra and Krishna 1981). The inability of wingless females housed with wingless males to deposit any fertile egg may presumably be due to failure in achieving proper and successful copula between such pairs. The marked fall in the laying of viable eggs by normal females subsequent to mating with males without wings clearly indicates that physical maiming of the male by excising its wings adversely affects the ability of this sex to properly inseminate the female during copulation. The relatively greater depressions in egg number and egg hatchability in females mated with hindwingless males in comparison to that recorded for counterpart mateds where sexual union took place with totally wingless males wherein also hindwings were severed are, indeed, interesting, if not intriguing. While it is decidedly premature at this stage to give any unequivocal explanation for such decrease in productivity, we feel it will not be entirely unreasonable to assume the occurrence of some sort of imperfect orientation, consequent to removal of only hindwings, by copulating males mounted on females causing the latter to get further poorly inseminated and thus to release fewer total and viable eggs.

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Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio*

SUMIT HOMECHAUDHURI, TAPAN PANDIT, SUBHAS PODDAR and SAMIR BANERJEE
Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India

MS received 20 January 1986

Abstract. Mahua oil cake, a derivative from the plant *Bassia latifolia*, is extensively used as fish toxicant cum pond manure in inland aquaculture systems of India. Saponin, one of the haemolytic components of mahua oil cake, has a lethal effect on fishes and other organisms. In the present investigation, *Heteropneustes fossilis*—an air-breathing catfish and *Cyprinus carpio*—a carp, were treated with mahua oil cake and their haematological indices were evaluated. The effect was fatally critical with gradual and complete shrinkage of erythrocytes, followed by ultimate haemolysis of cells, and significant reduction of the values of haematocrit, haemoglobin concentration and red cell count.

Keywords. Fish haematology; toxicology; effect of MOC; blood cells.

1. Introduction

Mahua oil cake (MOC), a derivative from the plant *Bassia latifolia*, is extensively used in aquaculture practices in India, both as a fish toxicant and an organic manure in fish ponds after its toxic effects are completely diminished. Most information regarding the effects of toxicity of MOC have been obtained through studies on the mortality rate (Lakshmanan 1983; Nath 1979). In the present communication, an attempt has been made to find out the changes in the blood parameters caused by the toxic effects of MOC. Bhatia (1970) reported that under laboratory conditions, the approximate threshold dose of MOC was 60 ppm. However, for complete eradication of predatory and weed fishes, MOC at 200–250 ppm is recommended by the Central Inland Fisheries Research Institute (CIFRI 1968) in India. According to Laksman (1983), susceptibility of fishes differed with species of fish (catla, common crap, tilapia and murrel were more susceptible to the cake than rohu, mrigal, magur and singhi, etc.). Hence, we selected the common crap, *Cyprinus carpio* and the catfish, *Heteropneustes fossilis* in order to compare their changes in blood values under the influence of MOC.

2. Materials and method

The study was carried out on sexually mature females of *H. fossilis* and *C. carpio* which were maintained for 48 h in plastic pools containing pond water. Powdered mahua oil cake at the prescribed dose of 250 ppm was applied to each of the pools and all the fishes were constantly observed to detect any visible morphological or behavioural changes due to poisoning.

The experiments were performed on several separate sets. In one, control studies were made, while in others, fishes were exposed to mahua oil cake. The carps were seen to be affected early with visible signs of poisoning and they were removed and blood tests were made after 30, 45 and 60 min of the application of MOC. Likewise, the catfishes were tested after 90, 105, 150, 165, 210 and 240 min of exposure to MOC.

Total erythrocyte count (TEC) was made using Neubauer's double haemocytometer and Shaw's solution as diluting fluid with 1/200 dilution in the red blood cell (RBC) pipette. Haemoglobin (Hb) concentration was estimated by acid haematin method and expressed in g/100 ml blood. Haematocrit (Ht) values were measured in the Wintrobe's microhaematocrit tubes filled with blood and centrifuged at 3000 rpm for 20–25 min. The blood smears were prepared and stained with Giemsa solution to study the shape and measurement of size of the erythrocytes of both the control and treated specimens. All the treated specimens were tested in distressed condition just prior to death. All the physicochemical parameters were also studied every hour using standard methods to find out any possible changes which might affect the species. To make an uniform comparison between the control and treated values, only females of both the species were selected.

3. Results

Observations on the behaviour of fishes on application of MOC indicate that the fishes tend to become gradually inactive and lose their balance. All the fishes in distinctly distressed condition come to the surface time and again, as if to get over breathing trouble and ultimately lie at the bottom. One result show that *C. carpio* float on the surface and *H. fossilis* sink to the bottom just before death. The experiment was repeated thrice and the results have been pooled (tables 1–5).

Table 1. Lethal effects of MOC on the blood values.

	C. carpio		*H. fossilis*	
Parameters	Control ± SD	Treated ± SD	Control ± SD	Treated ± SD
Length (range) cm	17·5–19·0	17·5–19·0	16·5–18·5	16·5–18·5
Weight (range) g	90·0–110·0	90·0–110·0	28·0–38·0	28·0–38·0
TEC ($\times 10^6/mm^3$)	2·12 ± 0·03	2·01 ± 0·01	2·35 ± 0·21	2·04 ± 0·05
Hb (g%)	9·7 ± 0·08	9·01 ± 0·13	10·7 ± 2·01	8·91 ± 0·24
Haematocrit (%)	35·38 ± 0·38	23·58 ± 0·48	37·53 ± 1·40	32·55 ± 0·92
Sex	Females	Females	Females	Females

Table 2. Gradual toxicity of MOC on the blood of *C. carpio*.

	Length (range) cm	Weight (range) g	Sex	Duration of treatment (min)	TEC ($\times 10^6/mm^3$)	Hb (g%)	Ht (%)
Control	15–17	80–100	♀	30	2·02	9·5	30·64
				45	2·03	9·4	31·67
Treated	15–17	80–100	♀	30	1·96	9·3	25·00

Table 3. Gradual toxicity of MOC on the blood of *H. fossilis*.

	Length (range) cm	Weight (range) g	Sex	Duration of treatment h	Duration of treatment min	TEC ($\times 10^6$/ mm^3)	Hb (g%)	Ht (%)
Control	16·2–21·2	25–50	♀	1	30	2·57	10·4	38·20
				1	45	2·95	13·0	38·00
				2	30	2·34	9·8	37·30
				2	45	3·17	13·8	40·50
				3	30	2·11	9·4	37·14
Treated	16·2–21·2	25–50	♀	1	30	2·52	9·8	35·00
				1	45	2·32	10·2	33·30
				2	30	2·09	9·1	33·30
				2	45	2·57	11·4	38·45
				3	30	2·01	9·0	30·00

Table 4. Changes in size and shape of erythrocytes.

Specimen	Duration of treatment	Shape of RBC	Size of RBC µ	Size of RBC ±SD	Remarks
C. carpio	Initial	Round/elliptical	18·86	3·35	Normal size and shape
	30 min	Irregular	12·45	3·04	Shrinkage of RBC
	60 min	Disintegrated	–	–	Complete haemolysis
H. fossilis	Initial	Round/elliptical	15·52	2·28	Normal size and shape
	2 h 30 min	Irregular	11·35	1·75	Shrinkage of RBC
	4 h	Disintegrated	–	–	Complete haemolysis

Table 5. Different physico chemical parameters during experiment.

	Duration of treatment (h) Initial	1	2	3
Air temperature (°C)	31·8	32·7	32·8	32·6
Water temperature (°C)	28·4	29·6	29·8	30·2
pH	8·25	8·30	8·37	8·35
Dissolved oxygen (ppm)	4·40	6·24	6·40	6·72
Methyl orange alkalinity (ppm)	460·04	472·76	468·52	461·10
Specific conductivity (mhos $\times 10^{-3}$)	2·37	2·40	2·33	2·43
Dissolved organic matter (mg Cl^{-1})	10·08	18·58	18·37	17·53

Free CO_2 (ppm) in all cases was found in traces.

3.1 *Lethal effects of MOC*

C. carpio females (length 17·5–19·0 cm and weight 90–100 g) seemed to be dying after exactly 60 min of treatment. In treated specimens TEC ($2{\cdot}01\times10^6\pm0{\cdot}01$), Hb content ($9{\cdot}01\pm0{\cdot}13$) and Ht value ($23{\cdot}58\pm0{\cdot}48$) were considerably low compared to the control specimens TEC ($2{\cdot}12\times10^6\pm0{\cdot}03$), Hb ($9{\cdot}7\pm0{\cdot}08$) and Ht ($35{\cdot}38\pm0{\cdot}38$) (table 1).

Female *H. fossilis* (length 16·5–18·5 cm and weight of 28–30 g) were in a state of coma after 3 h 45 min of treatment. In this case too, the treated specimens showed appreciable decrease in their blood values-TEC ($2{\cdot}04\times10^6\pm0{\cdot}05$), Hb ($8{\cdot}91\pm0{\cdot}24$) and Ht ($32\pm0{\cdot}92$), compared to that of control specimens TEC ($2{\cdot}35\times10^6\pm0{\cdot}21$), Hb ($10{\cdot}7\pm2{\cdot}01$) and Ht ($37{\cdot}53\pm1{\cdot}40$) (table 1).

3.2 *Gradual toxicity of MOC on blood*

The female specimens of *C. carpio* (length 15–17 cm and weight of 80–100 g) were studied after 30 and 45 min of treatment with MOC.

3.2a *After 30 min*: Treated specimens showed the values of TEC ($1{\cdot}96\times10^6$), Hb (9·3) and Ht (25·0), much lower than the control values of TEC ($2{\cdot}02\times10^6$), Hb (9·9) and Ht (31·67) (table 2).

3.2b *After 45 min*: Treated values of TEC ($1{\cdot}67\times10^6$), Hb (9·0) and Ht (18·75), were significantly lower than the control values of TEC ($2{\cdot}03\times10^6$), Hb (9·4) and Ht (31·67) (table 2).

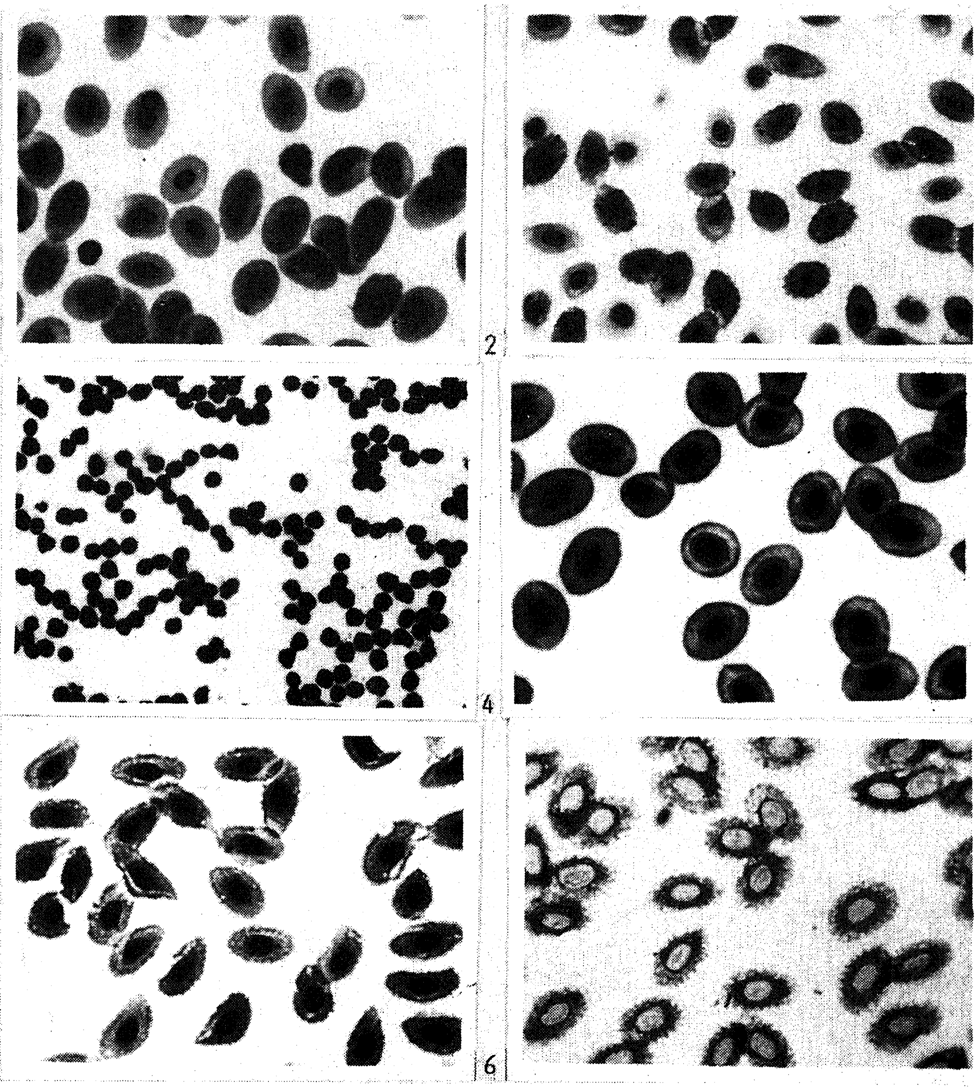
The females of *H. fossilis* (length 16·2–21·2 cm and weight 25–50 g) were studied after 90, 105, 150, 165, 210 and 240 min. In all the studies, it was evident that the TEC, Hb and Ht values in the species gradually decreased with time under the influence of MOC (table 3).

The shape of erythrocytes became irregular due to toxicity of MOC in both species and ultimately RBCs were subjected to a high degree of shrinkage and disintegration. In *C. carpio*, the normal size of erythrocytes ($18{\cdot}86\,\mu\pm3{\cdot}34$) was diminished to ($12{\cdot}45\,\mu\pm3{\cdot}04$) after 30 min of treatment (figure 5). In *H. fossilis*, the normal size of RBC ($15{\cdot}52\,\mu\pm2{\cdot}28$) decreased to the size of $11{\cdot}35\,\mu\pm1{\cdot}75$ after treatment for 2½ h (table 4) (figures 1–3).

The physicochemical parameters of water remained almost unchanged except dissolved organic matter (DOM). Initially, it increased from 10·08–18·58 mgCl^{-1} but slowly decreased to 17·53 mgCl^{-1} after 3 h of treatment.

4. Discussion

According to earlier reports (Hickling 1962; Bhatia 1970), MOC contains 4·6% of saponin, a fish toxicant which is known to be haemolytic in reaction. MOC enters the circulation through gills and buccal epithelium. The gradual shrinkage and ultimate haemolysis of RBC and subsequent alteration of the blood values of both *C. carpio* and *H. fossilis* could be understood from this fact. As is known, if



Figures 1–6. Photomicrographs of stained blood smear of *H. fossilis* (**1–3**) and *C. carpio* (**4–6**). **1 and 4.** Normal erythrocytes. **2 and 5.** Shrinkage of erythrocytes, breakage of red cell membrane after exposure to MOC. **2.** 1 h 30 min. **5.** 30 min. **3 and 6.** Complete disintegration of erythrocytes after exposure to MOC. **3.** 3 h 45 min. **6.** 60 min.

haemoglobin, instead of being encased within the corpuscles remains free in the plasma due to haemolysis, many ill-effects are bound to be produced. The following may happen: (i) The viscosity of blood will rise. (ii) Loss of haemoglobin will critically reduce the oxygen-carrying capacity of blood, thus producing anoxia and acidocis. (iii) Possibility of haemoglobinuria condition where the Hb would be excreted through urine, as free urine has easy access into the glomerular membrane of kidney tubules as a result of which a serious disturbance in the kidney functioning will be caused. (iv) Acute anaemia is a distinct possibility. All these deleterious effects depend upon the number of RBC haemolyzed and the rapidity of haemolysis.

We can conjecture from our experimental results that the toxicity of MOC played havoc very rapidly on the blood of both the species and led to all these abnormalities which became fatal. The gradual shrinkage and ultimate disintegration of RBC might have contributed to the poorer value of RBC count and Ht values. Poikilocytosis of RBC was observed immediately after treatment for a few hours (table 4). Initially, the RBC count was much low, perhaps because of the erythropoiesis stimulated by a decrease in oxygen content of blood due to dimunition of the amount of haemoglobin. The disintegration of RBC further enhances the possibility of acidocis due to loss of available surface area of erythrocytes which plays a pivotal role in maintaining the acid base balance and ion balance in the blood.

The water analysis, however, revealed that the physicochemical parameters remained mostly unchanged. The initial increase of DOM must be due to the high solubility of MOC contents. Although the dissolved oxygen content as in earlier reports (Laksman 1983) has increased to some extent, no inducive factor can be attributed to it.

It is interesting to note that *C. carpio* was more easily affected by MOC, compared to air-breathing *H. fossilis* which seemed to withstand the toxicity for a longer time as it takes oxygen from air. The blood values of *C. carpio* changed abruptly in response to the critical state due to poisoning.

Bhatia (1970) reported possible haemolysis of blood due to toxic actions of mahua cake, but this observation seemed to be confusing since the studies were made from dead test fishes. From our experimental results, we fully endorse the views regarding strong haemolytic action of MOC on live fishes.

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Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy

S L CHOUBISA
Department of Zoology, College of Science, Sukhadia University, Udaipur 313 001, India

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Abstract. Histochemical method for non-specific esterases has been applied to cercariae belonging to 4 different trematode groups viz Monostome, Amphistome, Xiphidio and Echinostome. Various structures containing esterases revealed positive reactions to the standard method and are visualized in simple whole mount preparation. Different degrees of non-specific esterase activities were observed in the nervous and the excretory systems, suckers and sub-tegumentary cells. In general, nervous systems of cercariae are composed of a pair of cerebral ganglia connected to each other by a transverse commissure to form a cerebral complex. These give off 3 pairs of anterior longitudinal and 3 pairs of posterior longitudinal nerve cords. Simultaneously nervous systems of redia of monostome cercaria have also been focused in the present study.

Keywords. Esterase; trematode; cercariae; redia; nervous system.

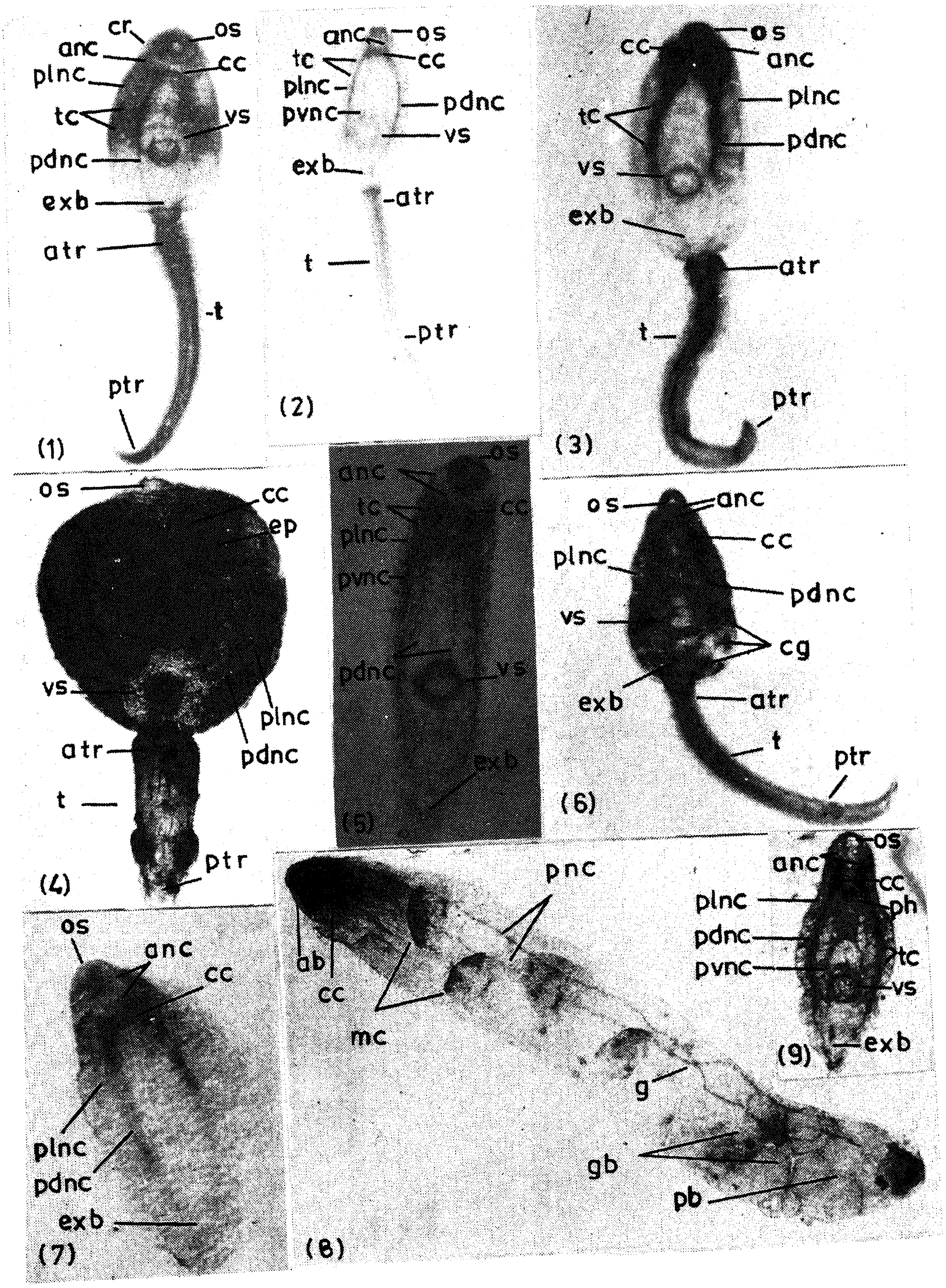
1. Introduction

Several workers have studied the nervous system by localization of either non-specific esterase (NSE) or acetylcholinesterase in different adult (Simha and Rao 1977; Rao *et al* 1981; Choubisa *et al* 1982; Mishra and Tandon 1984) as well as in certain larval digeneans (Dixon and Mercer 1965; Fripp 1967; Jennings and Leflore 1972; Bruckner and Voge 1974; Diconza and Basch 1975; Krishna and Simha 1979; Leflore 1979; Leflore *et al* 1980; Grabda and Moczon 1981; Venkatanarsaiah 1981; Choubisa and Sharma 1983b). None of these workers have attempted a comparative study on the nervous system of different larval trematode groups.

In the present study an attempt has been made not only to trace the nervous systems but also to observe the activity of NSE in different structures of cercariae and compare the time periods required for optimum visualization of esterases in 8 different cercarial species. The study also emphasises the nervous system of redia of monostome cercaria, *Cercaria buckleyi* (Pandey and Agrawal 1977).

2. Materials and methods

During the survey of larval digeneans and their snail hosts from freshwater habitats of Udaipur region (Rajasthan), different kinds of cercariae such as Monostome, Amphistome, Echinostome and Xiphidio were collected (Choubisa and Sharma 1983a; Choubisa 1985). The rearing of snails and method for collection of their trematode larvae have already been described (Choubisa and Sharma 1983a, 1985). For the present study 8 cercarial species viz *Cercaria komiyai* (figure 1), *Cercaria* sp. VII Kerala (figure 2), *C. granulosa* (figure 3), *C. pigmentata* (figure 4), *C. talensis* (figure 5), *Cercaria* sp. VIII Kerala (figure 6), *C. buckleyi* (figure 7) and *C. indicae*



Figures 1–9. **1.** *C. komiyai.* **2.** *Cercaria* sp. VII Kerala. **3.** *C. granulosa.* **4.** *C. pigmentata.* **5.** *C. talensis.* **6.** *Cercaria* sp. VIII Kerala. **7.** *C. buckleyi.* **8.** Redia of *C. buckleyi.* **9.** *C. indicae* XVII (× 100).
(ab, Anterior body; anc, Anterior nerve cord; atr, Anterior tail region; cc, Cerebral complex; cg, Cystogenous glands; cr, Collar region; exb, Excretory bladder; g, Ganglion; gb, Germ balls; mc, Mature cercaria; os, Oral sucker; pdnc, Posterior dorsal nerve cord; ph, Pharynx; plnc, Posterior lateral nerve cord; pnc, Posterior nerve cord; ptr, Posterior tail region; pvnc, Posterior ventral nerve cord; t, Tail; tc, Transverse connections; vs, Ventral sucker).

XVII (figure 9) and redia (figure 8) of monostome cercaria, *C. buckleyi* have taken. These larvae belonging to different groups of trematode have been listed in table 1. The standard techniques were used for the localization of NSE in cercariae and rediae as described by Holt and Withers (1952), and Jennings and Leflore (1972). Control whole mounts were also made out according to Choubisa and Sharma (1983b). Simultaneously, time periods were also noted for optimum visualization of NSE for each cercarial species.

3. Observations

NSE activity was seen in the form of deep blue microcrystals in nervous and excretory systems, sub-tegumentary cells, suckers, pharynx and in tail region of all 8 cercarial species. Not all but few cercariae, *C. indicae* XVII (figure 9), *C. komiyai* (figure 1) and *Cercaria* sp. VIII Kerala (figure 6) revealed the mild activity of NSE in their intestinal caeca. Cystogenous gland cells also showed strong activity of esterase in the *Cercaria* sp. VIII Kerala only. In general, the maximum activity of NSE was found on oral and ventral suckers and their adjoining areas, nervous system and in tail region, whereas excretory and digestive systems and genital rudiments showed moderate to mild activity of NSE. The various degree of enzyme activity in different regions of cercariae have been shown in table 1.

In the nervous system of all 8 cercarial species, the reaction of NSE was strong particularly on cerebral ganglia and pair of anterior and posterior dorsal longitudinal nerve cords. Lateral and ventral nerve cords revealed comparatively low activity. However, oral and ventral suckers also showed strong activity of esterase. It was clear from the variety of structures visualized by this reaction that a number of types of esterase were involved. Further, it was confirmed that use of inhibitor (10^{-4}M eserine sulphate) in the incubation medium, most of the cholinesterase activity in the nervous system was eliminated in the control cercariae and only cerebral ganglia were found slightly positive. No effect of the inhibition could be seen

Table 1. Activities of non-specific esterases in various structures of certain trematode larvae.

Types of larval trematode/ Larval species	OS	VS	Ph	Oes	C	CGC	GR	EB	NS	T	Incubation time (min)
Monostome											
Cercaria buckleyi	+ +	0	+	−	−	−	−	±	+ + +	+	5
Amphistome											
C. pigmentata	+	+	+	+	±	+ +	−	+	+ + +	+	12
Echinostome											
C. komiyai	+ +	+ +	+	+ +	±	+ +	−	+	+ + +	+ +	8
C. granulosa	+ + +	+ +	±	−	−	+	−	−	+ + +	+ + +	8
Cercaria sp. VII Kerala	+ +	+ +	+	−	−	±	−	+	+ + +	+ +	10
Cercaria sp. VIII Kerala	+ + +	+ +	+	+	+	+ + +	+	+ +	+ + +	+ +	7
Xiphidio											
C. talensis	+ +	+ +	+ +	−	−	+	−	±	+ + +	+	5
C. indicae XVII	+ + +	+ + +	+ +	+	+	±	+	±	+ + +	+	6

+ + +, Very intense; + +, moderate; +, mild; ±, faint; −, no activity; O, organ absent; C, caeca; CGC, cystogenous gland cells; EB, excretory bladder; GR, genital rudiments; NS, nervous system; OS, oral sucker; Ph, pharynx; T, tail; VS, ventral sucker.

in the other structures of cercariae such as alimentary canal, subtegumentary cells which appear to be scattered throughout the mesenchyme, posterior part of the excretory bladder, cystogenous cells, those cells in the oral and ventral suckers and central core of the tail. It has proved that number of esterase involved in these structures. Cercariae incubated in substrate free medium did not reveal the esterase activity in any tissues.

The nervous systems of present cercariae are found to be almost similar in pattern and composed of two cerebral ganglia connected with a thick band like transverse commissure to form a cerebral complex and lies immediately posterior to oral sucker, 3 pairs of anterior longitudinal and 3 pairs of posterior longitudinal nerve cords that arise from the lateral sides of each cerebral ganglia (figures 1–7 and 9). Three pairs of nerve cords could be differentiated according to their position, lateral, ventral and dorsal in the anterior and posterior regions, respectively. The anterior dorsal, ventral and lateral nerves and their numerous fine nerve branches encircle and innervate the oral sucker, pharynx and prepharynx regions. The posterior dorsal longitudinal nerve cords are the largest. They are stout, prominent and deeply stained compared with the posterior, ventral and lateral nerve cords. These, on their way towards the ventral sucker innervate the gut, genital rudiments, excretory bladder and also gives fine branches to the tegument. Each posterior lateral nerve cord units with its fellow, dorsal and ventral nerves by transverse connectives to form 6–12 transverse commissures in ring form immediately after commencement of the posterior region of cercarial body. Each commissure give off several fine branches of nerves distributed all over the body in the form of a net work.

In the tail region, all cercaria revealed strong activity of NSE particularly in the central core of the tail. Except *C. granulosa* (figure 3) none of the other cercarial species showed a pair of nerves, dorsal and ventral originating from the ganglion situated at near the base of tail and running posteriorly. 12–15 transverse connections in ring forms were also found in tail of this echinostome cercaria.

The nervous system of redia (figure 8) was found simple and composed of cerebral complex which lies immediately behind the pharynx. Pair of nerves originates from cerebral complex, proceeds posteriorly and unite to form a ganglion near three-fourth of the hind region of the body (figure 8). From this ganglion, again a pair of nerves runs to the posterior end of the redia. Cerebral complex also gives rise to 2–3 nerve branches which innervate the pharynx. No transverse connections were observed in these redia as found in its cercaria, *C. buckleyi*.

Different time periods were observed for optimum visualization of NSE activity in trematode larvae (table 1), 12 min for amphistome (*C. pigmentata*), 7–10 min for echinostome cercariae (*C. komiyai*, *C. granulosa* and *Cercaria* sp. VII and VIII Kerala), 5–6 min for xiphidio cercaria (*C. talensis* and *C. indicae* XVII), 5 min for monostome cercaria (*C. buckleyi*) and 15 min for redia of *C. buckleyi*.

4. Discussion

The localization of NSE in the larval trematodes is very helpful in tracing of many structures besides the nervous system. Jennings and Leflore (1972) have shown the various organ systems of cercaria of *Himasthala quissetensis* by localizing NSE in whole mount preparation. Similarly, Choubisa and Sharma (1983b) and Choubisa (1985) have also localized NSE in *Tetracotyle metacercaria, T. lymnaei* and in different cercarial groups viz *C. chauhani* (Monostome), *C. itoi* (Echinostome), *C. johrii* (Gymnocephalous), *C. thapari* (Xiphidio), and *C. udaipuriensis* and *C. gurayai* (Furcocercous) and discussed the various morphological features and their nervous systems. Simultaneously Choubisa (1985) has also recorded the various time periods for optimum visualization of NSE. The maximum incubation time, (14 min) was recorded for gymnocephalous cercaria, *C. johrii* and minimum (5 min) for *C. chauhani* (Monostome) and interpreted that former species showed a number of incipient adult features, such as the presence of immature gonads, and thus the occurrence of a more impermeable tegument. Similarly, in the present study amphistome cercaria, *C. pigmentata* revealed the maximum incubation time (12 min) and it can corroborate the findings of Choubisa (1985).

Earlier workers reported the presence of a nervous system in cercarial body but they could not define the arrangement of nerves in the tail region. However, Choubisa (1985) traced out the fine arrangement of nerves in the tail region of furcocercous cercaria, *C. udaipuriensis* and *C. gurayai*: two ganglia one situated at the anterior and other at posterior region where the tail is bifurcated. Two nerves, ventral and dorsal, originated from the anterior ganglion proceeded posteriorly and finally connected to the posterior ganglion. Further, both main nerves connected by 8–10 fine transverse connections of nerves in ring forms. Similarly, experimental cercaria, *C. granulosa* (figure 3) also revealed the nerve arrangement in its tail stem but other cercarial species did not show this clearly. However, the nervous system of these cercariae is basically similar but their other fine branches of nerves are differently distributed and it is related to activity of particular part of the body.

The nervous system of the redia in these studies (figure 8) is simple and composed of two cerebral ganglia united with each other by a transverse commissure to form a mass of cerebral complex situated behind the pharynx. One posterior nerve from each cerebral ganglion proceeds to three-fourth of redial body where both nerves are united to form a small ganglion and again a pair of nerves originate from it to run to the posterior end of the body. However, the findings of Krishna (1981) reveal that the nervous system of the redia *Echnistoma revolutum*, is composed of only a cerebral mass located behind the pharynx but no other nerve arrangement in redia could be observed. Other workers, Bruckner and Voge (1974) have also demonstrated the nervous system of miracidia of *Schistosoma mansoni* by means of ASChI and composed of a thick neural mass in the anterior region and 3 pairs of longitudinal nerve trunks, connected to the transverse commissure and it was almost similar to its cercaria. But nervous system of present redia is different from its monostome cercaria, *C. buckleyi* (figure 7).

In the present study, all different species of cercariae revealed the nervous system basically similar but arrangement and maximum of nerves in particular region of larvae were found to be different and it is correlated with the activity of particular regions such as oral and ventral suckers and also tails of certain cercariae, echinostome species (figures 1, 2, 3 and 6). Difference in the incubation time periods for optimum visualization of esterase activity in various cercariae depend on the permeability of tegument and stage of their development.

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Growth and population dynamics of the Indian white prawn *Penaeus indicus* H M Edwards from Kakinada

S LALITHA DEVI
Kakinada Research Centre of Central Marine Fisheries Research Institute, Kakinada 533 002, India

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Abstract. The von Bertalanffy parameters of growth in *Penaeus indicus* are estimated as $L_\infty = 218{\cdot}9$ mm, $K = 2{\cdot}0$ per year, and $t_0 = 0{\cdot}01$ year in females and 227·2 mm, 1·8 per year and $-0{\cdot}0729$ year, respectively in males. The instantaneous rates of mortality are estimated as $Z = 9{\cdot}42$, $M = 3{\cdot}30$ and $F = 6{\cdot}12$ in females and 12·73, 2·90 and 9·83, respectively in males. Various morphometric and length-weight relationships are worked out. At the current rate of fishing, the yield in weight per recruit is 23·05 g in females and 31·74 g in males. There is scope to increase the yield of this species by increasing F (i.e. effort).

Keywords. Growth; mortality rates; yield per recruit.

1. Introduction

Kakinada is an important prawn landing centre in Andhra Pradesh. Due to great demand for prawns in the export trade, the fishing industry has expanded tremendously in recent years by continuous addition of trawlers. Prawns are also fished in the inshore waters and backwaters by indigenous gear. They form about 25% of the total fish catch and among them, *Penaeus indicus* forms about 8·2%. While Kurup and Rao (1975) studied the growth and mortality rates from Ambalapuzha, west coast of India, there is no information on the growth and population dynamics of *P. indicus* from anywhere along the coasts of India. The results of a study on these aspects based on the data collected during 1979–1982 from the landings of commercial trawlers at Kakinada and the backwater landing centres are presented in this paper.

2. Materials and methods

Data on effort and catch of prawns were collected for 15–20 days in a month at the Kakinada fisheries harbour and once in a week at the backwater landing centres of Kakinada (Matlapalem, B V Palem and Pedagadimoga, 10–32 km southeast of Kakinada). Effort standardisation was made following Gulland (1968), in terms of Pomfrets. Details of the craft and gear were given by Muthu *et al* (1977). In the backwaters stakenets and dragnets were operated. Total length (from tip of rostrum to tip of telson) and individual weight of prawns were recorded to the nearest mm and mg, respectively. Throughout this study, males and females were treated separately. The data on the weight of *P. indicus* landed and its length composition were collected at weekly intervals; these data were raised to get the monthly estimates. Growth was studied by the progression of modes in the monthly length frequency distribution of the catch, and the parameters of growth in length were imated using the von Bertalanffy equation which is of the form

$$L_t = L_\infty\{(1-\exp[-K\ (t-t_o)])\},$$

ere L_∞ = asymptotic length, K = a constant equivalent to 1/3rd of catabolic efficient and t_0 = arbitrary origin of the growth curve.

The values of L_∞ and K were estimated using the Ford-Walford plot (Ford 1933; alford 1946) on the basis of the lengths attained at intervals of two months. The ue of t_0 was estimated by a plot of $\text{Log}_e\ (L_\infty - L_t)$ against t. The values of K and t_0 is obtained were converted into those of per year and year, respectively. The total gth-carapace length relationship was calculated by using the equation $Y = a + bx$ edecor and Cochran 1967).

The length-weight relationship was calculated using the equation (Le Cren 1951).

$$\log W = \log a + b \log L,$$

ere W is weight in grams and L is length in mm. The range in total length of nales and males was 48–204 and 50–216 mm, respectively.

Instantaneous rate of total mortality (Z) was estimated for each year following the ch curve of Pauly (1983). The natural mortality rate (M) was estimated using the ation $Z = M + qf$. It was also estimated taking the life span ($T_{\max}$) of the species in : fishery and assuming that 99% by numbers would die by the time they attain $_{ax}$ in an unexploited state (Sekharan 1975; Cushing 1981).

The yield in weight per recruit (Y_w/R) was calculated using the simplified Beverton d Holt's (1957) formula as given by Ricker (1958).

$$\frac{Y_w}{R} = F\left[\exp[-Mt_c - t_r]\right] W_\infty \left[\frac{1}{Z} - \sum_{n=0}^{3} \frac{U_n(\exp[-nk(t_c - t_0)])}{F+M+nK}\right]$$

The value of W_∞ was estimated by taking the value of L_∞ and the constants of al length-total weight relationships. The value of length corresponding to the first int in the descending straight portion of the catch curve was taken as the length at t capture, and the age corresponding to this length was taken as t_c; t_r is the age at ich the prawn becomes vulnerable to some common type of fishing. The data lected from the backwaters was used only to study the growth in early stages of *P. icus.*

Results

Estimation of growth parameters

a *Length frequency analysis of the backwater catch*: Data collected during 1979 d 1980 are presented in figure 1. As there is continuous recruitment and as the wns live for a short period in these nursery grounds, before they migrate to the , modal progression for a given mode could not be traced for more than 3 months. : length ranged from 20–120 mm.

b *Females*: Mode *A* at 56·5 mm in January 1979 is traceable to 106·5 mm in rch. Mode *B* at 46·5 mm in February 1979 could be connected to the mode at 5 mm in March. Mode *C* at 56·5 mm in March is taken to have grown to 86·5 mm

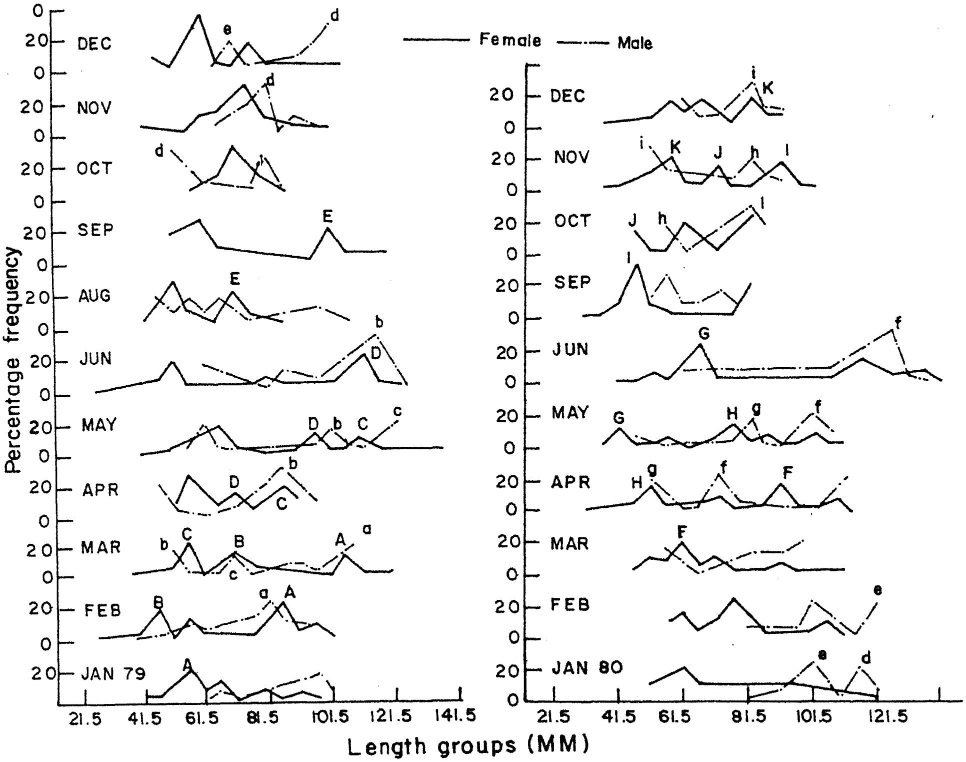

Figure 1. Monthly length frequency distribution of *P. indicus* during 1979–'80 (backwater catch).

in April and to 111·5 mm in May. Mode *D* at 71·5 mm in April 1979 is traceable to 111·5 mm in June. Mode *E* at 71·5 mm in August 1979 can be connected to 101·5 mm in September. Mode *F* at 61·5 mm in March 1980 is taken to have grown to 91·5 mm in April, mode *G* at 41·5 mm in May 1980 is traceable to 66·5 mm in June and mode *H* at 51·5 mm in April 1980 to 76·5 mm in May. Mode *I* at 46·5 mm in September can be connected to the mode at 91·5 mm in November. Likewise mode *J* at 46·5 mm in October 1980 is traceable to 71·5 mm in November and mode *K* at 56·5 mm in November 1980 is taken to have grown to 81·5 mm in December.

The above observations indicate an average growth rate of 25–30 mm/month.

3.1c *Male*: Mode *a* at 81·5 mm in February 1979 is traceable to 111·5 mm in March, mode *b* at 51·5 mm in March 1979 can be connected to the mode at 101·5 mm in May and to 116·5 mm in June. Mode *c* at 71·5 mm in March 1979 is taken to have grown to 121·5 mm in May. Mode *d* at 51·5 mm in October 1979 is traceable to 81·5 mm in November, and to 116·5 mm in January 1980. Mode *e* at 71·5 mm in December 1979 is taken to have grown to 101·5 mm in January 1980, and to 121·5 mm in February 1980. Mode *f* in April 1980 at 71·5 mm can be connected to the mode at 101·5 mm in May and to 126·5 mm in June. Mode *g* at 51·5 mm in April 1980 is traceable to 81·5 mm in May. Mode *h* in October 1980, at 56·5 mm is taken to have grown to 81·5 mm in November and mode *i* at 51·5 mm in November 1980 is traceable to 81·5 mm in December.

The above observations indicate 35 mm monthly increase in length between 51 mm and 86 mm and 30 mm between 81 and 111 mm, which gives an average growth of 32 mm/month.

3.2 *Length frequency analysis of the marine catch*

Data collected during 1979–1981 are presented in figure 2. The length range of the females and males observed in the fishery was 48–204 and 51–216 mm, respectively.

3.2a *Females*: Mode *A* in February 1979 at 146·5 mm is traceable to 171·5 mm in April. Mode *B* at 126·5 mm in February 1979 can be connected to the mode at 151·5 mm in April. Mode B_1 at 96·5 mm in February is traceable to 141·5 mm in April. Mode *C* at 121·5 mm in May 1979 is taken to have grown to 161·5 mm in August. Mode *D* at 181·5 mm in September 1979 is traceable to 191·5 mm in November. Mode *E* at 121·5 mm in October 1979 can be connected to the mode at 166·5 mm in January 1980. Mode *F* at 111·5 mm in October 1979 is taken to have grown to 161·5 mm in February 1980. Mode *G* at 106·5 mm in January 1980 is traceable to 126·5 mm in February, mode *H* at 136·5 mm in February 1980 can be connected to the mode at 161·5 mm in April, mode *I* at 136·5 mm in June 1980 is taken to have grown to 166·5 mm in September; mode *J* at 96·5 mm in July 1980 can be connected to the mode at 136·5 mm in October, mode *K* at 116·5 mm in July 1980 is taken to have grown to 131·5 mm in August, mode *L* at 136·5 mm in September 1980 is traceable to 156·5 mm in November; mode *M* at 156·5 mm in September 1980 can be connected to the mode at 176·5 mm in November. Mode *N* at 81·5 mm in October 1980 is taken to have grown to 161·5 mm in April 1981, mode *O* at 121·5 mm in November 1980 is traceable to 161·5 mm in February 1981, mode *P* in March 1981 at 126·5 mm can be connected to the mode at 146·5 mm in May, mode *Q* at 111·5 mm in March 1981 is traceable to 131·5 mm in May; mode *R* at 96·5 mm in April 1981 to 176·5 mm in November, mode *S* at 91·5 mm in July, 1981 is taken to have grown to 131·5 mm in October and mode *T* at 81·5 mm in September 1981 is traceable to 121·5 mm in November.

The above observations indicate that the monthly growth rates are 20 mm between 81·5 mm and 121·5 mm, 12·5 mm between 126·5 and 151·5 mm, 10 mm between 156·5 mm and 176·5 mm and 5 mm between 181·5 and 191·5 mm. Since a growth of 25–30 mm/month is observed in the backwaters, individuals of the modal length 81·5 mm were taken as 2·9 months old.

3.2b *Male*: Mode *a* at 116·5 mm in January 1979 is traceable to 146·5 mm in April. Mode *b* at 121·5 mm in March 1979 can be connected to the mode at 136·5 mm in April, mode *c* at 91·5 mm in April 1979 is taken to have grown to 136·5 mm in June, mode *d* at 101·5 mm in May 1979 is traceable to 141·5 mm in August, mode *e* at 121·5 mm in September 1979 can be connected to the mode at 161·5 mm in November, mode *f* at 106·5 mm in December 1979 is taken to have grown to 136·5 mm in February 1980, mode *g* at 121·5 mm in December 1979 is traceable to 166·5 mm in February, mode *h* at 116·5 mm in March 1980 can be connected to the

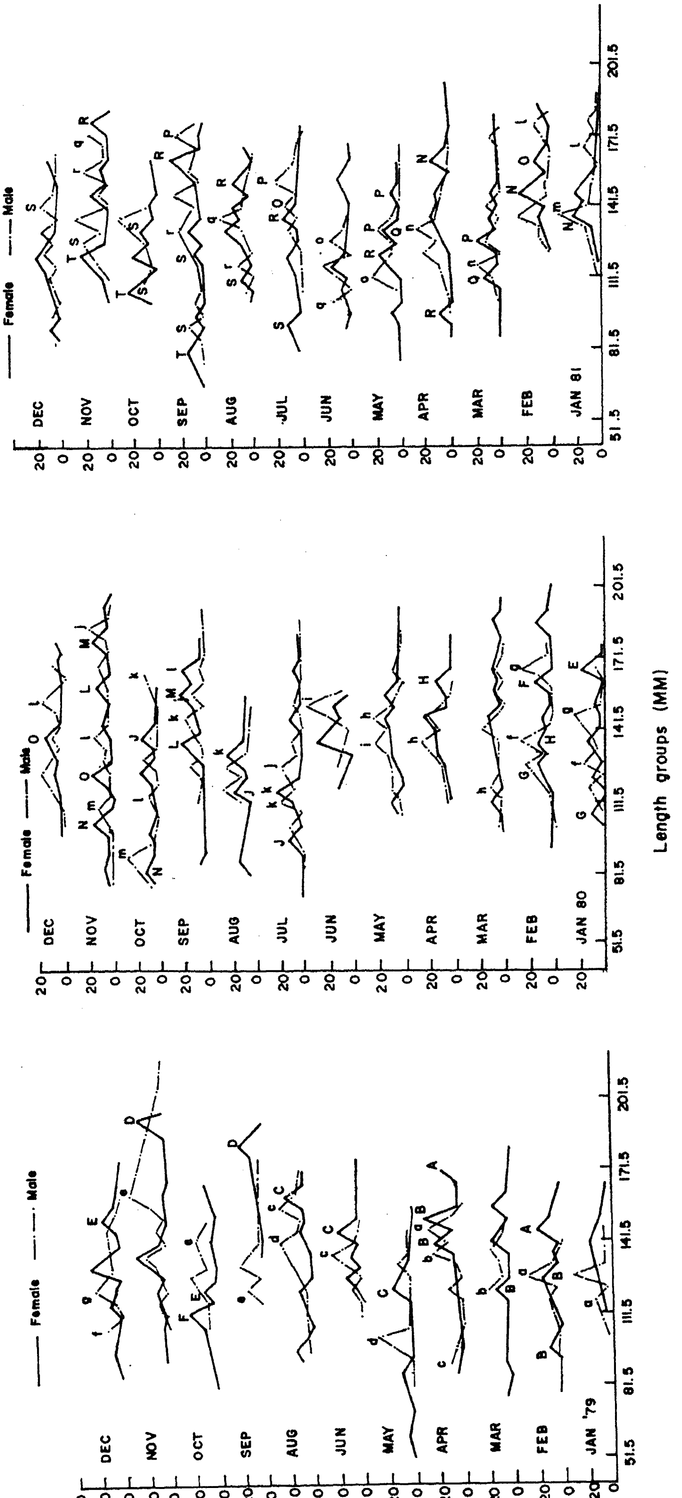

Figure 2. Monthly length frequency distribution of *P. indicus* during 1979–'81 (marine catch).

mode at 146·5 mm in May, mode *i* at 136·5 mm in May 1980 is traceable to 151·5 mm in June, mode *j* at 126·5 mm in July 1980 is taken to have grown to 181·5 mm in November, mode *k* at 111·5 mm in July 1980 is traceable to 161·5 mm in October, mode *l* at 111·5 mm in October 1980 can be connected to the mode at 176·5 mm in February 1981 mode *m* at 86·5 mm in October 1980 is traceable to 136·5 mm in January 1981, mode *n* at 116·5 mm in March 1981 can be connected to the mode at 131·5 mm in April, mode *o* at 111·5 mm in May 1981 is traceable to 141·5 mm in July, mode *p* at 131·5 mm in May 1981 is taken to have grown to 171·5 mm in September; mode *q*, at 101·5 mm in June 1981 is traceable to 171·5 mm in November, mode *r* at 116·5 mm in August 1981 can be connected to the mode at 156·5 mm in November and mode *s* at 91·5 mm in September 1981 is taken to have grown to 141·5 mm in December.

From the above observations, the monthly growth rates are 20 mm between 86·5 mm and 126·5 mm, 15 mm between 126·5 and 156·5 mm and 12·5 mm between 146·5 mm and 171·5 mm. Since a growth of 32 mm/month was observed in the backwaters, the prawns forming a mode at 91·5 mm can be reasonably taken as 2·9 months old.

Using the above growth rate and from the Ford-Walford plot (figures 3A, B) the values of L_∞ and K for females and males were estimated as 218·9 mm and 2·0 per year and 227·2 mm and 1·8 per year, respectively. The t_0 value was estimated as 0·01

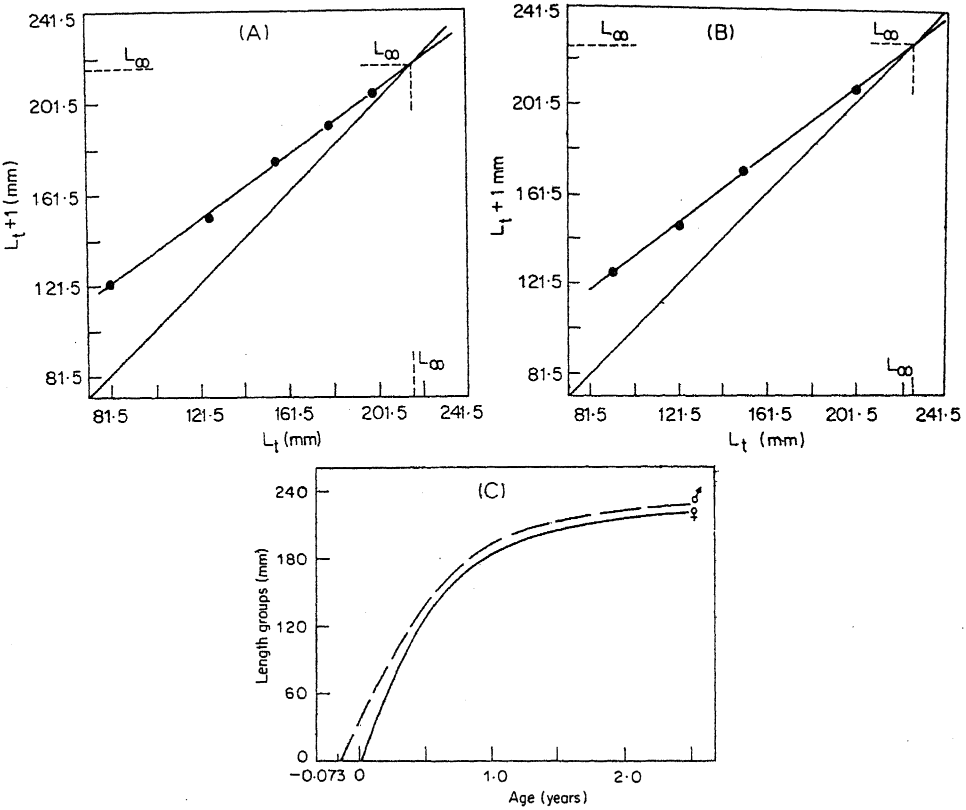

Figure 3. Ford-Walford plot in *P. indicus*. **A.** Female. **B.** Male. **C.** Theoretical growth curve in *P. indicus*.

year in females and −0·0729 year in males. The theoretical growth curves of male and female *P. indicus* are shown in figure 3C.

The maximum lengths of the male and female observed in the fishery are 216 and 204 mm, respectively. The age (T_{max}) of these prawns is calculated as 1·6 and 1·4 years respectively.

3.3 *Morphometric and length-weight relationships*

The parameter values of the regressions of various morphometric and length-weight relationships are presented in table 1. The regression lines between sexes in the total length-carapace length relationship showed significant difference (table 2). Comparison of slopes and elevations obtained for various length-weight relationships are not significant at 1% level. Hence the data of the sexes were pooled and combined equations were given.

3.4 *Estimation of mortality rates*

3.4a *Total mortality* (*Z*) (figure 4): In each year, the total mortality rate of males was

Table 1. Morphometric and length-weight relationships of *P. indicus*.

Relationship	Sex	N	a	b	r^2
Carapace length (Y) on total length (X)	F	66	1·559	0·3946	0·9976
	M	142	4·478	0·3679	0·9924
* Total weight (Y) on total length (X)	Data of the sexes pooled	104	−6·0438	3·4584	0·9500
* Head less weight (Y) on total length (X)	Data of the sexes pooled	177	−7·1896	3·9469	0·9308
Head less weight (Y) on total weight (X)	Data of the sexes pooled	340	0·0637	0·7645	0·9671
* Total weight (Y) on carapace length (X)	Data of the sexes pooled	340	−5·8635	4·1641	0·8998

*After logarithmic transformation.

Table 2. Analysis of co-variance to test the significance of differences between regression lines of sexes in the total length-carapace length relationship of *P. indicus*.

Sources of variation	Deviation	Fronr SS	Regression MS
Due to regression within sexes	204	97·3054	0·47698
Differences between regression co-efficients	1	25·9727	25·9727
Residuals due to regression pooled within	205	123·2781	0·60443
Differences between adjusted mean	1	67·87795	67·879
Total	206	191·15065	–

Comparison of slopes $F = 54·452$ (df 1,204) HS
Comparison of elevation $F = 112·3007$ (df 1,205) HS
HS, Highly significant at 1%

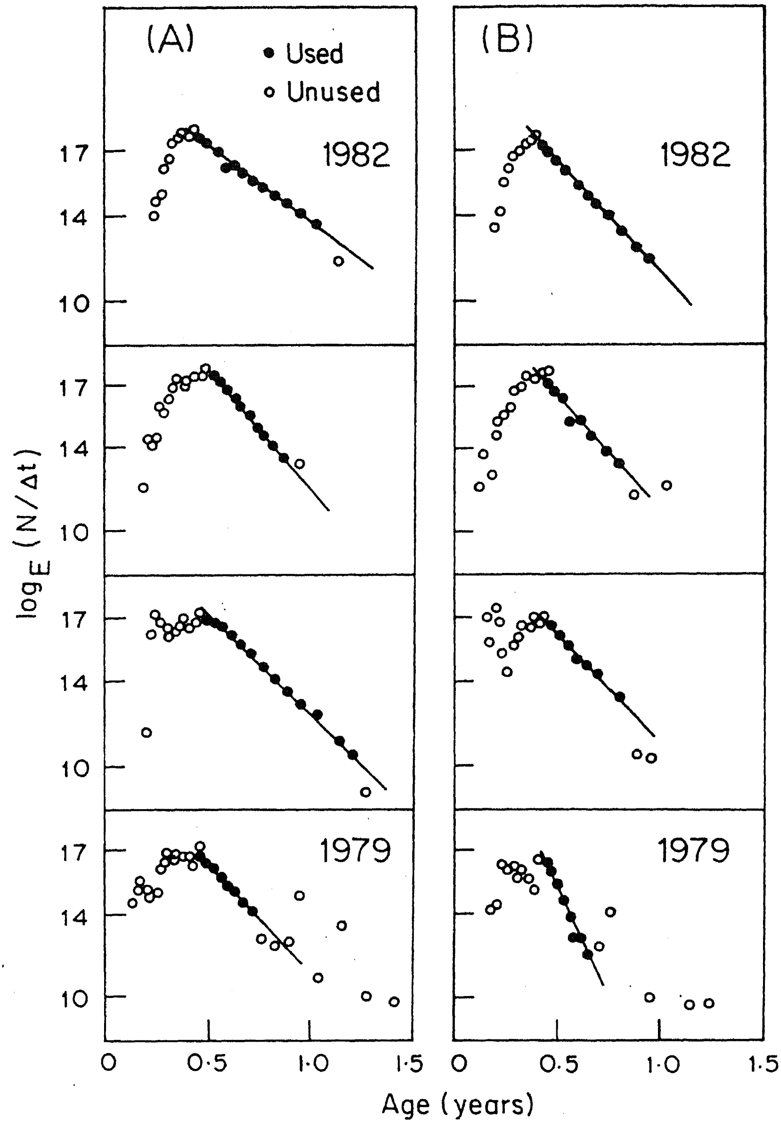

Figure 4. *P. indicus* estimation of Z by catch curve method of Pauly 1983. (**A**) Female; (**B**) Male.

greater than that of females except in 1981. The average for females and males was estimated as 9·42 and 12·73, respectively (table 3).

3.4b *Natural mortality rate* (M): Plot of Z values of males and females of *P. indicus* against the corresponding effort showed poor correlation ($r = 0{\cdot}083$ in females and 0·671 in males). Hence the M values obtained by taking the life-span into account were considered (table 3).

Table 3. Estimated values of mortality rates in *P. indicus*

	Total mortality rates	
Year	Females	Males
1979	9·95	20·45
1980	9·36	9·77
1981	11·36	10·61
1982	7·01	10·10
Average Z	9·42	12·73
Natural mortality rate (M)	3·30	2·90
Fishing mortality rate (F)	6·12	9·83

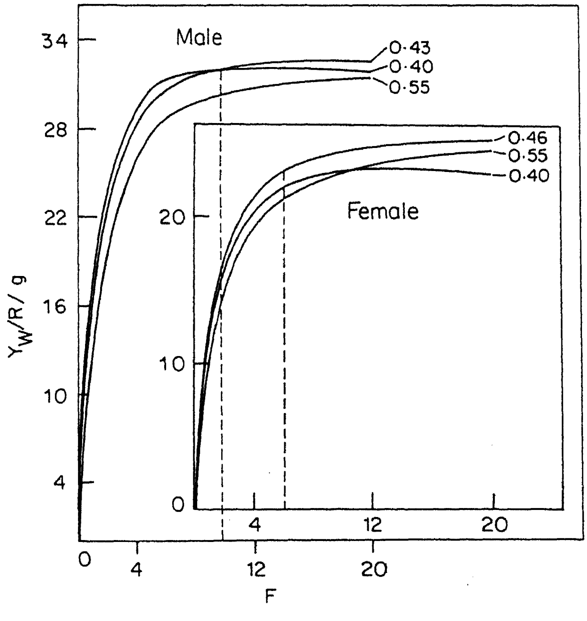

Figure 5. Yield in weight per recruit in *P. indicus*. Vertical lines show the present F. The numerals indicate the t_c in years.

3.5 *Yield per recruit*

Yield in weight per recruit as a function of fishing mortality rate F, for various t_c values are shown in figure 5. At present, F for females and males is 6·12 and 9·83, respectively. Present t_c is 0·46 year for females and 0·43 year for males. At the current rate of fishing, the yield per recruit is 23·05 g in females and 31·74 g in males. In both the sexes, the yield curves indicate increase in the yield with increase in F (i.e. effort). By keeping M constant and varying t_c, the yield curve in females indicates (figure 5) better yield at $t_c = 0·40$, but it is not advisable to reduce t_c to 0·40, as the age at which *P. indicus* attains first maturity is 0·42 year. Increase of t_c to 0·55 year, though results in a marginal decrease in the yield, but is desirable, since the prawn is allowed to spawn before it is fished. In males, increase in fishing effort with increase in selection size upto 0·55 year would give better yield.

4. Discussion

Observations on the length frequency analysis at the backwaters indicate a monthly growth rate of 30–35 mm (average 32 mm) in the case of males and 25–30 mm/month (average 27·5 mm) in females. These results are in agreement with those of Suseelan (1975), Sultan *et al* (1973) as mentioned by Mohammed (1970) and tag recovery experiments (Anonymous 1982) wherein the growth rates observed were 30·55 mm/month, 25·8 mm/month and 21–36 mm/month, respectively.

The values of the growth parameter K of *P. indicus* obtained in this study indicate that at Kakinada, the females attain the asymptotic length faster than males whereas Kurup and Rao (1975) observed faster growth in males than in females and females attained maximum length. Though there is disparity in the growth rates of males and females, the longevity of the male and female prawns are comparable.

The average annual total mortality rate, Z in males is greater than in females as is generally the case in penaeid prawns (Garcia and Le Reste 1981). Kurup and Rao (1975) estimated Z as 3·1 in males and as 2·1 in females. They did not estimate M. By taking into account, the growth parameters and the maximum length of females at 180 mm and males at 175 mm given by them, the $T_{\max}$ is estimated as 1·95 years in females and 1·3 years in males. Following the same method as adopted in the present work, the values of M were estimated as 3·5 for males and 2·4 in females. These M values thus obtained for the population in the Ambalapuzha area are slightly greater than the Z values estimated by these authors. According to Kurup and Rao (1975) ‘trawling for prawns in the offshore fishing grounds has also been introduced recently’ (p 184). Hence, the Z values obtained by Kurup and Rao (1975) can be reasonably taken as to represent M. The values of M obtained for *P. indicus* in the present study are comparable to those obtained for the same species from Ambalapuzha. Hence, the high values of Z from the Kakinada region can only be due to high fishing mortality. This is reasonable because the data pertain to the period 1979–1982 and trawling is carried out almost exclusively to catch prawns (Rao *et al* 1981).

It is known that estimation of M of a particular species in a multispecies fishery using the regression of Z against effort is difficult (Pauly 1983). This may be due to the difficulty in measuring the effective effort for each species. It is obviously for this reason the regression of Z against effort showed poor correlation in this study.

According to Le Reste (1978) and Marcille (1978), in *P. indicus*, the M values are 0·21/month and 0·15–0·25/month i.e. 2·52 and 1·8–3·0/year, respectively at Madagascar. They did not treat the sexes separately. It may be noted that the present average M (for both the sexes) worked out to 3·1 and the M/K ratio to 1·63 which is within the range given by Beverton and Holt (1959) and as quoted by Garcia and Le Reste (1981) for fast growing organism with short life span.

The yield per recruit analysis shows that at present the effort has no adverse effect on the stock and further increase in effort with increase in the selection size would give better yield.

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Prediction of assimilation efficiency of lepidopterans

T J PANDIAN and M PETER MARIAN
School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. From 206 values reported for over 60 lepidopteran species, it was found that nitrogen content of food shows a significant ($P < 0.001$) and a positive correlation ($r = 0.704$) to assimilation efficiency with standard error of 10·33. Unlike in aquatic animals, water content of food influences assimilation efficiency in these terrestrial lepidopterans; however, the relation between leaf water and assimilation efficiency is also correlated, but the correlation is lower than that ($r = 0.529$; SE = 11·8) obtained for leaf nitrogen. Considering the individual effect of water on assimilation efficiency as well as its covarying relation with nitrogen, leaf water was included as a co-predictor. The inclusion of leaf water as a co-predictor of leaf nitrogen content improves the precision of prediction ($r = 0.868$; SE = 9·7). The role of digestive reducing substances (e.g. Tannins) on assimilation efficiency was considered as a second co-predictor. Realising the difficulties of considering the digestive reducing substances as a second co-predictor, and observing the closeness of the observed and predicted values in the present study, nitrogen and water contents of leaf are considered as adequate explanatory variables for the prediction of assimilation efficiency.

Keywords. Assimilation efficiency; lepidopteran species; leaf water; digestive reducing substances.

1. Introduction

The ease and accuracy, with which faeces egested by the lepidopterous larvae can be estimated, have prompted several workers to choose either a component of faeces (e.g. Uric acid: Bhattacharya and Waldbauer 1969, 1970) or the whole of faeces (Mukerji and Guppy 1973; Mathavan and Pandian 1974) as an index of food consumption. Such an indirect procedure of estimation of food consumption is based on an assumption that assimilation efficiency (Ase) remains constant around 45%. Scriber and Slansky (1981) showed that nitrogen and water contents of food may significantly influence Ase of lepidopterans fed on juice, forb, root or wood. Pandian and Marian (1985a) observed that the constancy of Ase at 45% is limited for forb-feeding lepidopterans alone. Hence, there is a need to identify a marker for indirect estimation of Ase of lepidopterans feeding on juice, root or wood. More recently, Pandian and Marian (1985b, c, d, e) have found that nitrogen content of food is positively and significantly correlated to absorption efficiency of fishes, reptiles, aquatic insects and polychaetes and can be used as an index of absorption/assimilation efficiency in these animal groups; they have further shown that the scope for the applicability of their method is greater, and their method is more reliable than any other indirect procedure described thus (Pandian and Marian 1985b). Therefore, an attempt is made to explore the possibility of using nitrogen content of food as an index of Ase of lepidopterans.

1.1 *Prerequisite for prediction*

Basic information reported for Ase of over 66 species is summarized in table 1. An

Table 1. Ase of lepidopterous larvae fed on plants containing different nitrogen and water levels under different experimental conditions.

Species, stage and sex	T (°C)	Rh (%)	R (%)	Plant species	W (%)	N (% dry wt)	Ase (%)	Remarks	Reference
Danaus chrysippus		80	*ad lib*	*Calotropis gigantea*	84	3·0			Mathavan and Pandian (1975)
♂	19						57	T affects Ase	
♀	19						58		
♂	27						58		
♀	27						48		
♂	32						49		
♀	32						43		
♂	37						48		
♀	37						46		
D. chrysippus	30	80		*C. gigantea*	84	3·0			Mathavan and Muthukrishnan (1976)
	30		100				47	Food restriction affects Ase	
			50				46		
			25				41		
			Fed for 3, 6, 9, 12 and 24 h/d				43		
D. chrysippus		75		*C. gigantea*	85	3·0			Pandian *et al* (1978)
	20		*ad lib*				30	T and R affect Ase	
	25		*ad lib*				23		
	30		*ad lib*				28		
	35		*ad lib*				51		
	30		56				36		
	30		37				40		
	30		32				43		
	30		30				41		
	30		15				49		
	30		7				51		
D. chrysippus (I–V instar)	29	80	*ad lib*	*C. gigantea*					Marian and Pandian (1980)

				Young leaf	84	3·0	60	Old leaf reduces
				Old leaf	88	0·8	36	Ase
D. plexippus	27		*ad lib*	*Ascelpias syriaca*					Schroeder (1976a)
		85			77	4·0	60		
		85			77	3·6	47		
		82			76	3·2	46		
Lymantria dispar	22	35	*ad lib*						Barbosa (1978)
				Quercus rubra	2·3*	22			
				Q. alba	3·8*	47			
				Fagus grandifolia	2·2*	23			
				Acer rubrum	2·3*	22			
				Betula populifilia	3·8	30			
L. dispar			*ad lib*						Barbosa and Greenblatt (1979)
	24	90		*B. populifolia*	3·3*	30			
				F. grandifolia	2·2*	19			
				A. rubrum	2·3*	22			
				Q. alba	3·8*	47			
				Q. rubra	1·9*	23			
L. monacha	30		*ad lib*	Beech	60	2·0*	21		Sattler (1939)
Catopsilia crocale		81		*Cassia alata*	84	3·6			Christopher (1983)
	17		25				47	T and R affect Ase	
			50				52		
			75				54		
			100				61		
	22		25				50		
			50				52		
			75				54		
			100				60		
	27		25				50		
			50				50		
			75				51		
			100				54		
	32		25				50		
			50				49		
			75				49		
			100				52		

Table 1. (*Contd.*)

Species, stage and sex	T (°C)	Rh (%)	R (%)	Plant species	W (%)	N (% dry wt)	Ase (%)	Remarks	Reference
C. crocale (I–V instar)	30	85	*ad lib*	*C. alata*	84	3·8	50		Mathavan and Nambirajan (1976)
Polydorus aristolochiae	30	85	*ad lib*	*Aristolochia bractiata*	86	3·6	67		Haniffa *et al* (1982)
Bombyx mori	22	75	*ad lib*	mulbery	74	4·2*	42		Hiratsuka (1920)
B. mori			*ad lib*	mulbery	74	4·2*			Horie and Watanabe (1983a)
♂							42		
♀							42		
Tinea pellionella	20								Chauvin and Gueguen (1978)
		95	*ad lib*				52	Humidity affects Ase	
		5	*ad lib*				39		
Ostrinia nubilabis	26	75	*ad lib*	Wheat germ		2·5*			Hubner and Chiang (1982)
Healthy							36 ± 8	Parasitism does not affect Ase	
Parasitised							46 ± 15		
Diatraea saccharalis	26	75	*ad lib*	Wheat germ		2·5*			Hubner and Chiang (1982)
Healthy							40 ± 13		
Parasitised							50 ± 17		
Pericallia ricini (I to V instar)	28	85	*ad lib*						Krishnan (1984)
				Gossypium hirsutum	89	2·8	48		
				Raphanus sativus	90	2·7	50		
				Solanum melongena	85	2·2	47		
				Hibiscus rosasinensis	81	1·4	46		

					Hibiscus esculentus	76	1·1	44	
					Gossypium hirsutum	89	2·8	50	
Spodoptera eridania		22	35	*ad lib*					Scriber (1981)
	P				Dill	88	4·4	50	
	P				Bean	87	4·9	77	
	F				Dill	88	4·4	73	
	F				Cabbage	93	4·6	78	
S. eridania		22		*ad lib*					Scriber (1978b)
	P				*Phaseolus vulgaris*	88	5·6	75	
	F							73	
	P				*Lotus corniculatus*	88	4·3	68	
	F				4 wks old, CN+			45	
	P				4 wks old,	87	4·2	59	
	F				CN−			48	
	P				20 wks old, CN+	79	4·1	53	
	F				20 wks old, CN+			40	
	P				20 wks old, CN−	80	4·3	52	
	F				20 wks old, CN−			34	
S. eridania		22	75	*ad lib*	Corn				Manuwoto and Scriber (1982)
	P				B49	81	7·0	81	
	P				San Juan	87	5·8	79	
	P				DIMBOA-less	91	7·0	75	
	F				B49	90	6·3	58	
	F				San Juan	87	5·8	61	
	F				DIMBOA-less	90	6·4	64	

Table 1. (*Contd.*)

Species, stage and sex	T (°C)	Rh (%)	R (%)	Plant species	W (%)	N (% dry wt)	Ase (%)	Remarks	Reference
S. eridania	21		*ad lib*	Vernal alfalfa	83				Kingsley *et al* (1983)
				control		4·7	70 ± 3	No significant effect of parasitism	
				Verticillium infected	83	4·8	66 ± 2		
S. eridania			*ad lib*						Scriber (1982)
				Sorbus americana	65	3·5	46		
				Betula papyrifera	71	3·2	49		
				Prunus serotina	73	4·4	62		
S. eridania	22		*ad lib*	Maize					Scriber *et al* (1975)
				Low DIMBOA	92	4·7	71		
					92	4·9	68		
					91	7·0	68		
				High DIMBOA	89	2·0	52		
					92	3·7	59		
					92	4·1	73		
					91	7·0	81		
Spodoptera littoralis	27–33	56–62	*ad lib*						Duodu and Biney (1981)
				Urena		3·3*	35		
				Cotton		3·6*	41		
				Cabbage	93	4·6*	73		
S. litura			*ad lib*	*Zinnia elegans*	82	3·1*		T does not affect Ase	Chockalingam and Somasundaram (1983)
	25						40		
	30						40		
	35						50		

Pseudoplusia includens		27	60	*ad lib*	Soyabean	86	4·6		Life stage affects Ase	Kogan and Cope (1974)
	I							71		
	II							75		
	III							62		
	IV							43		
	V							53		
	VI							44		
								58 ± 14		
Pieris brassicae (II–V instar)					*Raphanus sativus*		3·1	78		Yadava *et al* (1979)
P. brassicae				*ad lib*	*Raphanus sativus*		3·6*	65		Vats *et al* (1977)
P. rapae		18–24	60–80	*ad lib*						Slansky and Feeny (1977)
					Cleoma spinosa	86	3·8	33		
					Hesperis matronalis	81	3·2	31		
					Barbarea vulgaris	82	2·8	35		
					Lunaria annua	88	3·7	26		
					Dentaria diphylla	88	4·4	38		
					Thlaspi arvense	87	4·6	43		
					Lepidium virginicum	83	3·7	29		
					Brassica oleracea	87	3·0	31		
					B. oleracea	85	2·1	26		
					B. oleracea	86	2·4	28		
					B. oleracea	87	2·5	34		
					B. oleracea	84	2·4	29		
					B. oleracea	84	1·9	28		

Species, stage and sex	T (°C)	Rh (%)	R (%)	Plant species	W (%)	N (% dry wt)	Ase (%)	Remarks	Reference
				B. juncea	88	3·8	33		
				B. nigra	87	4·8	41		
				B. perkinensio	90	2·9	36		
				Tropaeolum majus	86	3·0	42		
P. rapae	21	60–84	*ad lib*	*Brassica oleracea*	85	1·7			Slansky (1978)
Normal							41	No parasitic effect	
Parasitized							39		
Pachysphinx modesta	27	60–80	*ad lib*	*Populus deltoides*	53	1·6*	41		Schroeder (1973)
Euchaetias egle	27	87	*ad lib*	*Asclepias syriacea*	76	3·2	46		Schroeder (1977)
Papilio glaucus (III and IV instar)	20–24		*ad lib*						Scriber (1979a)
				Prunus serotina	61	2·1	29		
				Fraxinus americana	51	2·0	29		
				Sassafras albidum	68	3·0	36		
				Lindera benzoin	70	2·0	35		
				Sassafras albidum	68	3·0	24		
P. glaucus			*ad lib*	*Prunus serotina*	66	3·2			Scriber and Lederhouse (1983)
P	15						34		
P	22						44		
F	22						32		

	P	26						37		
	F	26						32		
	P	30						41		
	F	30						55		
	P	37						37		
	F	37						47		
Hyalophora cecropia		27	70	*ad lib*	*Acer negundo*		2·7	37		Schroeder (1971)
H. cecropia		20–20	60–90		*Prunus serotina*	88	3·7		Food restriction does not affect Ase	Grabstein and Scriber (1982a)
				Fed for 24h/d				33		
				16h/d				34		
				16h/ after 4 days				33		
				16h/ after 5 days				28		
H. cecropia				*ad lib*	Black cherry	67	3·1	36		Grabstein and Scriber (1982b)
					Paper birch-Black cherry	68	3·1	37		
						62	2·3	27		
						63	2·3	26		
H. cecropia		22	75	*ad lib*						Scriber (1977)
					Prunus serotina	70	2·8	32	Water does not affect Ase	
						59	2·8	31		
						55	2·6	34		
					Quercus alba	49	2·7	37		
Calocalpe undulata		27	80		*Prunus serotina*	62	3·3			Schroeder (1976b)
				Fed for 24 h/d				33	Food restriction increase Ase	
				20 h/d				34		
				16 h/d				36		

Table 1. (*Contd.*)

Species, stage and sex	T (°C)	Rh (%)	R (%)	Plant species	W (%)	N (% dry wt)	Ase (%)	Remarks	Reference
Operophthera brumata	15		*ad lib*						Axelsson *et al* (1975)
(II–V instar)				Hazal leaf		3·0*	41		
(II–V instar)				Oat leaf		3·1*	42		
Mamestra configurata	25	70	*ad lib*						Bailey (1976)
				Potato		3·7*	52		
				Lambsquarters		3·7*	51		
				Rape (Zephyr)		3·6*	51		
				Rape (Span)		3·7*	50		
M. configurata	25	70	*ad lib*	*Brassica napus*		3·0*	40		Bailey and Singh (1977)
Achaea janata (II–V instar)		80	*ad lib*	*Ricinus communis*	84	3·8			Muthukrishnan and Pandian (1983)
	22						63	T and life stage affect Ase	
	27						60		
	32						60		
	35						64		
A. janata		80		*R. communis*	84	3·8			Muthukrishnan and Pandian (1984)
	22		30				67		
			46				62	T and R affect Ase	
			56				61		
			73				59		
			78				61		
			100				58		
	27		24				61		
			35				58		
			48				57		
			53				58		
			74				59		

			100				58	
	32		37				57	
			54				61	
			64				60	
			74				63	
			77				63	
			100				63	
	37		38				58	
			49				60	
			52				61	
			65				62	
			86				64	
			100				63	
Protoparce sexta			*ad lib*	Tobacco		3·5*	43	Waldbauer (1964)
Diacrisia virginica	27	78	*ad lib*	*Prunus serotina*	55	3·2	34	Schroeder and Malmer (1980)
Hyphantria cunea					46	3·2	38	
Bapta vestiliata					56	3·2	39	
Scopula sp.					65	4·2	50	
Hydria prunivorata					60	3·4	36	
Malacasoma americana					68	4·3	53	
Phobetron pithecum					55	3·0	31	
Hemerocampa leucostigmata					59	3·5	41	

T, Temperature; Rh, relative humidity; R, ration (% of *ad libitum*); W, water content of food; N, nitrogen content of food; P, penultimate instar; F, final instar.

*Values obtained from Sreeramulu (1982); Varma *et al* (1982); Winton and Winton (1939).

ncisive analysis of the table reveals the following: (i) The publications by Kogan and Cope (1974), Axellson *et al* (1975), Yadava *et al* (1979), Marian and Pandian (1980) and Krishnan (1984) are the only 5 reports available on the effect of life stage on Ase; all these authors have uniformly reported that the life stage significantly affects Ase; for instance, the efficiency of *Danaus chrysippus* decreases from 90% in the first instar to 58% in the final instar (Marian and Pandian 1980). (ii) The publications by Mathavan and Pandian (1975), Pandian *et al* (1978), Muthukrishnan and Pandian (1983, 1984), and Chockalingam and Somasundaram (1983) are the only 5 reports available on the effect of temperature on Ase; the first 4 reported that temperature significantly affects Ase, while the fifth contradicted the observation. (iii) The publications by Pandian *et al* (1978), Mathavan and Muthukrishnan (1976), Schroeder (1976b), Grabstein and Scriber (1982a) and Muthukrishnan and Pandian (1984) are the only 5 reports available on the effects of ration on Ase; a recalculation of their data revealed that of these, the first 4 indicated that ration significantly affects Ase, whereas the fifth one contradicts this observation. (iv) A couple of other publications ndicated that humidity prevailing in the terrarium and water content of food may or may not affect Ase. Apparently, life stage definitely affects Ase, whereas the effect of the remaining factors on Ase is questionable. Since some or all these factors may or may not significantly alter the expected linear relation between food N and Ase, it is a necessary prerequisite to identify the factors, which may significantly alter the expected relation. Such an approach is necessary to improve the prediction of Ase of epidopteran. To evaluate the magnitude of each one of these factors on the Ase, we have chosen to study basic information reported by Krishnan (1984).

Krishnan (1984) carried out a series of experiments on the moth *Pericallia ricinii*: he larva of the moth passes through 5 instars before pupation; all feeding experiments were undertaken from hatching to prepupation, exposing adequate number of individuals to a range of temperature (26–35°C), ration (25–100% of *ad*

Table 2. ANOVA to test the effect of different variables on Ase of lepidopterans. For combinations 1, 2 and 3, basic data were obtained from Krishnan (1984). For combination 4, data were selected from table 1.

Variable	*F*	*P*
Combination		
1. *Nitrogen × life stage × ration*		
Nitrogen	$F(1)4, 240 = 45$	$< 0{\cdot}005$
Life stage	$F(1)2, 240 = 205$	$< 0{\cdot}0005$
Ration	$F(1)3, 240 = 5$	$< 0{\cdot}01$
2. *Temperature × life stage*		
Temperature	$F(1)3, 80 = 37$	$< 0{\cdot}001$
Life stage	$F(1)4, 80 = 244$	$< 0{\cdot}0005$
3. *Temperature × Nitrogen*		
Temperature	$F(1)2, 71 = 16$	$< 0{\cdot}05$
Nitrogen	$F(1)2, 71 = 151$	$< 0{\cdot}0005$
4. *Water content × nitrogen*		
Water	$F(1)2, 4 = 4$	$< 0{\cdot}001$
Nitrogen	$F(1)2, 4 = 12$	$< 0{\cdot}05$

libitum), food quality with reference to different levels of nitrogen (1·1–2·8%). Thus Krishnan reported adequate information for suitable statistical analysis (ANOVA) to identify magnitude of the effects of the chosen parameters on Ase. The results obtained from two and three-way ANOVA tests conducted in different combinations of the variables nitrogen vs life stage vs ration: temperature vs life stage: temperature vs nitrogen: water content vs nitrogen on the Ase are summarized in table 2. It is understood from the table that all the variables are significantly ($P<0·05$) influencing the Ase; however, the magnitude of influence varies from one variable to other, which is inferred from the F values. Among these variables, the magnitude of influence of life stage on Ase is the greatest ($F \geqslant 205$), i.e. it is 5, 7 and 41 times more than that of nitrogen, temperature and ration, respectively. Food nitrogen has the next greatest magnitude of influence on Ase; its level of effect on Ase is 9, 9 and 3 times greater than that of temperature, ration and water content of food, respectively. On the whole, the level of influence on Ase by the tested factors is in the following order: life stage > food nitrogen > food water > temperature > ration.

Hence, it is chosen to include only the Ase values reported for the final instar in all the ensuing analyses for prediction of Ase. In a few cases, it was also possible to consider the total amount of ingestion and egestion from hatching to prepupation; in such cases, the mean Ase values were included (e.g. *Danaus chrysippus*, Marian and Pandian 1980). Such a prerequisite is justified for following reasons: (i) of information available for 66 species (table 1), Ase values are reported only for final instar of 61 species (table 1); (ii) most lepidopterans consume over 75% of the larval food during the final instar (Pandian and Delvi 1973; Pandian 1973) and (iii) the chances of errors introduced in estimating Ase in the first few instars are more, as the amount of ingestion or egestion is so little; for instance, the first instar *D. chrysippus* consumes 2·3 mg and egests 0·47 mg on the first day (Marian and Pandian 1980).

2. Materials and methods

Pertinent publications, which appeared during the period from 1920–1984, served as the basic material for the present study. From a survey over 240 publications were considered. Of these about 178 publications were not selected for the following reasons: (i) Lack of information on N content of food (e.g. Schowalter *et al* 1977); (ii) vague information on N content (e.g. Mukerji and Guppy 1970); (iii) reporting information on Ase, following the method of Mathavan and Pandian (1974), which assumes Ase to remain constant around 45% (e.g. Mathavan *et al* 1984); (iv) reporting desired information for insects reared under abnormal stress conditions such as exposure to pesticides (e.g. Chockalingam and Krishnan 1984) and other chemicals (e.g. caffeine and theophylline: Muthukrishnan *et al* 1979).

We have chosen about 60 publications reporting reliable information on total assimilation efficiency (in terms of dry weight or energy) of final instar fed on natural food under normal, healthy and experimental conditions. In some cases, it has been possible for us to secure nitrogen content of food, for which desired information is reported elsewhere (e.g. Sreeramulu 1982). Efforts were made to give due representation to forb and tree-leaf feeding lepidopterans so that the entire range of food nitrogen spectrum would be represented. Information thus obtained was analysed under the following heading (table 1): (i) species, stage and sex, (ii) temperature, relative humidity and ration, (iii) water and nitrogen contents of food and (iv) Ase:

$$\text{Ase}(\%) = \frac{\begin{array}{c}\text{Food ingested—faeces}\\ \text{(mg dry wt or energy)}\quad\text{(mg dry wt or energy)}\end{array}}{\begin{array}{c}\text{Food ingested}\\ \text{(mg dry wt or energy)}\end{array}} \times 100$$

Statistical analyses were done following Zar (1974), and computations were done using IBM 1130 computer.

3. Results

Available information on Ase of lepidopterans is presented in table 1; it reveals that (i) of over 60 species, more than 36 are moths and the remaining are butterflies; (ii) life stage of some tested species ranged from hatchling (e.g. *Pseudoplusia includens*: Kogan and Cope 1974) to final (upto VI) instar; (iii) nitrogen content of the tested food ranged from 0·8% (e.g. senescent *Calotropis gigantea* leaf; Marian and Pandian 1980) to 7% (e.g. maize B49 varieties; Manuwoto and Scriber 1982); (iv) water content of the food ranged from 49% (e.g. *Quercus alba*; Scriber 1977) to 93% (e.g. cabbage; Duodu and Biney 1981); (v) some species were given different rations; 7% (e.g. *D. chrysippus*; Pandian *et al* 1978) to 100% (e.g. *Pieris brassica*: Yadava *et al*

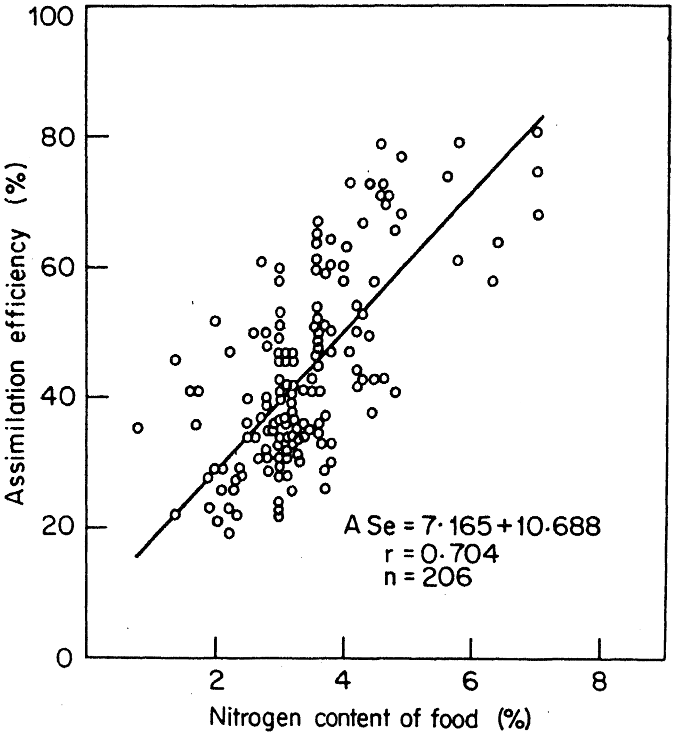

Figure 1. Assimilation efficiency of lepidopterans as a function of nitrogen content of food.

1979) of *ad libitum*; (vi) few temperate species were experimented at a temperature of 15°C (e.g. *Operohthera brumata*; Axelsson *et al* 1975) and (vii) the lowest Rh recorded during the experiment was 5% and the maximum 95% (e.g. Chauvin and Gueguen 1978). Thus it has been possible to summarize data on assimilation efficiency of a number of lepidopterans tested under a range of feeding regimes and experimental conditions.

Figure 1 illustrates the relationship between nitrogen content of food and Ase of the final instar of the selected lepidopterans. The Ase was the lowest (19%) for *Lymantria dispar* fed *ad libitum* on *Fagus grandiflora* containing 2·2% N at 24°C (Barbosa and Greenblatt 1979). With increasing food-N, the efficiency increased linearly to 81% in the armyworm *Spodoptera eridania*, which was fed on the corn B49 containing 7% N (Manuwoto and Scriber 1982). It is obvious that the nitrogen content of food is related to the Ase in lepidopteran larvae.

The relationship between the two variables was tested by computing the available data ($N = 206$) using simple regression model. The regression (Ase = 7·165 + 10·688 N) obtained with 0·704 correlation coefficient was statistically highly significant ($F = 200{\cdot}3$; $P < 0{\cdot}0005$) but the standard error (SE) of the estimate was high (10·33) (table 2). Hence, there is vast differences between the observed and predicted values, when food-N alone is used as predictor of Ase (table 8).

In general terrestrial animals acquire water mainly from food, and gain or lose water through body surface (Tracy 1976). Restriction of ration or exposure to higher temperature may result in dehydration, and force lepidopterous larvae to replenish water from food by accelerating the feeding and alimentation processes; such an acceleration may lower the Ase. Hence it is very likely such factors like water content of food, ration, temperature and Rh may modify the simple linear relationship observed between food-N and Ase, and may be responsible for the wide scatter of Ase values around the regression line fitted for the relation between food-N and Ase. Clearly, these 4 independent variables besides food-N may play a significant or negligible role in the determination of Ase. To understand the magnitude, to which the said 5 independent variables affect the dependent variable, Ase, hundred values were randomly selected from table 1. These values were used for correlation matrix analysis and the computed values are presented in table 4. The correlation values obtained for Ase for ration, temperature and Rh are very low, indicating that their effect on Ase is negligible. Moreover, the obtained matrix is singular; hence further computation of these 3 variables is neither possible nor required. The values obtained for nitrogen and water contents of food are equally high that it is difficult to identify from this analysis which has greater influence on Ase. To identify this, 164 values representing the relationship between water content of food and Ase were selected from table 1. The relation between these two variables is positive and linear but the Ase values are widely scattered at any chosen water level. In other words the situation recalls that observed for food-N-Ase relation, confirming the observation of Mattson (1980). He observed that water and nitrogen contents of food usually covary positively and make it difficult to comprehend the individual and combined effects of these variables on Ase.

An attempt was made to understand the magnitude of independent-effect of nitrogen and water contents of food on Ase. For this a partial correlation matrix was computed considering 145 observed values from table 1. The partial correlation values obtained for Ase clearly show the overriding importance of nitrogen over

Table 3. Summary of the simple regressions and related statistics computed for the relationships between the two independent variables (nitrogen, water) and the dependent (Ase) variable.

Simple regression equation	Correlation coefficient (r)	Standard error of estimate	F value of ANOVA	Level of significance (P)
Ase (%) = 7·165 + 10·688 N	0·704	10·328	F(1) 1,204 = 200·4	< 0·0005
Ase (%) = − 0·311 + 0·598 W	0·529	11·8	F(1) 1,162 = 62·8	< 0·0005

Table 4. The correlation matrix obtained for the relationships between the independent variable, i.e. Nitrogen (N), Water (W) content of food, Ration (R), Temperature (T), or Relative humidity (Rh) and dependent variable, i.e. Assimilation efficiency (Ase) of lepidopterans. The original values were obtained from those presented in table 1.

	N	W	R	T	Rh	Ase
N	1·00000					
W	0·08678	1·00000				
R	0·04216	0·32398	1·00000			
T	0·05860	0·12107	0·07234	1·00000		
Rh	0·06534	0·19381	0·07662	0·07007	1·00000	
Ase	0·51596	0·51031	0·10398	0·00428	0·00504	1·00000

Table 5. Matrix of partial correlation coefficients computed for the relation between the independent variable, i.e. Nitrogen or water content of food and dependent variable. Ase of selected Lepidopterans (for original values, refer table 1).

Variable	Nitrogen	Water	Ase
Nitrogen	1·00000		
Water	0·33223	1·00000	
Assimilation efficiency	0·69281	0·50928	1·00000

water in determining the efficiency (table 5). Still it is also apparent that water also exert a recognizable influence in determining the Ase, as the value obtained for water is not too low (0·51) in comparison to that (0·69) obtained for nitrogen. Therefore one has to include water as a co-predictor to improve the accuracy of prediction.

For this, a multiple regression was computed (Ase $= a + b_1 N + b_2 W$) considering a model, in which water is included as a co-predictor of food-N (table 6). When relevant data were fitted into this model, the correlation coefficient value was considerably improved ($r = 0{\cdot}868$) with 9·7 SE. In other words, the multiple regression thus obtained is Ase = − 11·610 + 8·246 N + 0·509 W. Since the overall SE of the regression model is 9·7, it is necessary to give some examples to show the reliability of the model (table 7). For this, examples were randomly selected from table 1 representing the entire range of nitrogen (0·8–7·0%) as well as water (49–92%) contents of the food. The observed and predicted values, in which water served as a co-predictor of food-N, are closer to each other (table 8) than those predicted considering food-N as a sole predictor.

Table 6. Regression statistics computed for the model: $\text{Ase}=a+b_1\text{N}+b_2\text{W}$ fit to the data presented in table 1.

Variable	Mean	Standard deviation	Correlation x vs y	Regression coefficient	Std. error of reg. coef.	Computed 't' value
Independent						
Nitrogen	3·53515	1·04585	0·69281	8·24567	0·81966	10·05978
Water	76·80000	12·70910	0·50928	0·50891	0·06745	5·36218
Dependent						
Ase	45·31724	14·65176				
Intercept		− 11·61021				
Multiple correlation (R^2)		0·75336				
Std error of estimate		9·70268				

Table 7. Analysis of variance for the regression computed for the model $\text{Ase}=a+b_1\text{N}+b_2\text{W}$

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F value
Attributable to regression	2	17545	8772	93
Deviation from regression	142	13368	94	
Total	144	30913		

4. Discussion

The term Ase is equivalent to 'Approximate Digestibility' (AD) of (Waldbauer 1968) or the 'coefficient of digestibility' of House (1965). It refers to the percentage of ingested food transferred through the gut into the lumen of insect body. It is generally calculated from the estimates of ingestion (I), egestion (E), i.e. faeces and excretary products (FU). Unlike in aquatic animals, the recovery and quantification of FU of lepidopterans are easier and complete, but the quantitative estimates of I is laborious and time-consuming, requiring the following gravimetric estimates: (i) weights of the offered food and the unfed remains, (ii) weight of transpiratory loss from the offered leaves and (iii) water content of the aliquot for each leaf offered, as each leaf of the same plant species varies considerably (Pate 1975). Considering the transpiratory water loss and photosynthetic production in the offered food leaves, Axelsson and Agren (1979) and Wightman (1981), respectively suggested several measures to improve the accuracy of the method, which however, involve additional estimates. To avoid the difficulties involved in the quantitative estimates of I, indirect methods were devised. However, the applicability of indirect marker methods like the chromic oxide (Cr_2O_3) (e.g. Horie and Watanabe 1983b) in phytophagous lepidopterans is limited and questionable; pointing out the differential passage of Cr_2O_3 in the digestive tract from that of feed. Bowen (1979) questioned the validity of Cr_2O_3 as a marker in the study of food assimilation. Park and Kogan (1981) have also pointed out that Cr_2O_3 interfere with food consumption. Thus, there is a need to identify an easily measurable component of food, which could serve as a reliable index of Ase of lepidopterans.

Table 8. Observed and predicted values of assimilation efficiency of selected Lepidopterans.

Species	Food	Water content of food (%)	Nitrogen content of food (%)	Assimilation efficiency (%) Predicted from N	N&W	Observed	Reference
Danaus chrysippus	*Calotropis gigantea*	80	3·0	39	54	51	Mathavan and Pandian (1975)
D. chrysippus	*C. gigantea* (young)	84	3·0	39	56	60	Marian and Pandian (1980)
D. chrysippus	*C. gigantea* (senescent)	88	0·8	16	40	36	Marian and Pandian (1980)
Spodoptera eridania	DIMBO A. less mutant maize	92	4·7	57	74	71	Scriber *et al* (1975)
Polydorus aristolochiae	*Aristolochia bractiata*	86	3·6	45	62	67	Haniffa *et al* (1982)
Achaea janata	*Ricinus communis*	84	3·8	48	63	63	Muthukrishnan and Pandian (1983)
S. eridania	Corn variety 1	81	7·0	82	87	81	Manuwoto and Scriber (1982)
S. eridania	Corn variety 2	87	5·8	69	81	79	Manuwoto and Scriber (1982)
Hyalophora cecropia	*Quercus alba*	49	2·7	36	36	37	Scriber (1977)
Hyphantria cunea	*Prunus serotina*	46	3·2	41	39	38	Schroeder and Malmer (1980)

Nitrogen content of animals is usually higher than that of plants; animals consist mainly (>50%) of protein (Hafez and Dyer 1969; DeFolliart 1975) or nitrogen (7·14%); plants consist mainly of carbohydrates and their nitrogen content ranges from 0·03–7% (Mattson 1980), averaging to 2% nitrogen (Russell 1947). Nitrogen requirements of animals is far higher than that of plants, as the former use proteins as structural building-blocks, whereas the latter use carbohydrates. Besides, animals also use nitrogen less efficiently than plants, as a significant fraction of daily wastes consists of various nitrogen compounds, whereas plants excrete little nitrogen (Mattson 1980). Consequently herbivores such as lepidopterans must consume, sequester and assimilate as much nitrogen from food stuffs as possible; if the process of assimilation is 50% efficient, then most lepidopterans must eat 4–5 units of food stuff to obtain adequate nitrogen to make one unit of their body tissue. Hence food plants containing low nitrogen are likely to be assimilated less efficiently so that more food is consumed to procure adequate nitrogen; the reverse would be true for the plants with high nitrogen. Thus it is appropriate that nitrogen, a non-inert moiety of the food is considered as an index of Ase of lepidopterans. Pandian and Marian (1985d) found that nitrogen content of food nitrogen was significantly ($P < 0{\cdot}001$) and positively correlated ($r = 0{\cdot}97$) to Ase of aquatic insects as well as in several other animals groups and it has been possible for them to predict Ase from food nitrogen of several animal groups with less than 10% error (Pandian and Marian 1985b, c, d, e). Hence our attempt to predict Ase of lepidopterans using food nitrogen is likely to hold good.

However, it must be noted that whereas the correlation coefficients (r) obtained for food-N and Ase relationship were higher than 0·9 for other animal groups, that obtained for lepidopterans is far less ($r = 0{\cdot}7$) and involves a higher SE too. The low r and high SE obtained for lepidopterans reflect the wide scattering of Ase data against food-N (figure 1). Such wide scattering of Ase data indicates the influence of other factors like water and digestibility reducing substances of the food plants. The relationship between N content of leaf and Ase, and the effect of water as a co-explanatory variable in the determination of Ase of lepidopterans have also been documented by Rausher (1981) in the pipevine swallowtail butterfly *Battus philenor* fed with two host plants, *Aristolochia reticulata* and *A. serpentaria*. The F value of Rausher's multiple regression was less (F 1, 26 = 14·6) than that reported in the present study (F 1, 145 = 93). Despite the variation in the F values, the common concept inferred is that both N and water contents of a food plant interact to determine the digestibility or Ase of lepidopterans; among the two, N displayed an overriding importance over water content of leaves in the determination of Ase. Incidentally, McNeill and Southwood (1978) and Morrow and Fox (1980) have also observed a positive and significant correlation between food-N and Ase in non-lepidopteran insects, i.e. *Leptopterus dolabrata* (Homoptera) and *Parapsis atomaria* (Coleoptera). Feeding 22 lepidopteran species belonging to Papilionidae and Bombycoidae on the leaves of different food plants (trees and forbs), Scriber (1978a) found that larvae fed on low water content tree leaves exhibited low Ase, whereas those fed on forbs exhibited relatively higher Ase. A glance over figure 2, in general confirms the observation of Scriber that leaf water content of trees is in general lower than the forbs. However, leaf water content of some trees (e.g. *Cassia alata*; Christopher 1983) is as high as that of some forbs and *Catopsilia crocale* feeding on *C. alata* leaf displays as high Ase as those feeding on forbs containing 85% water

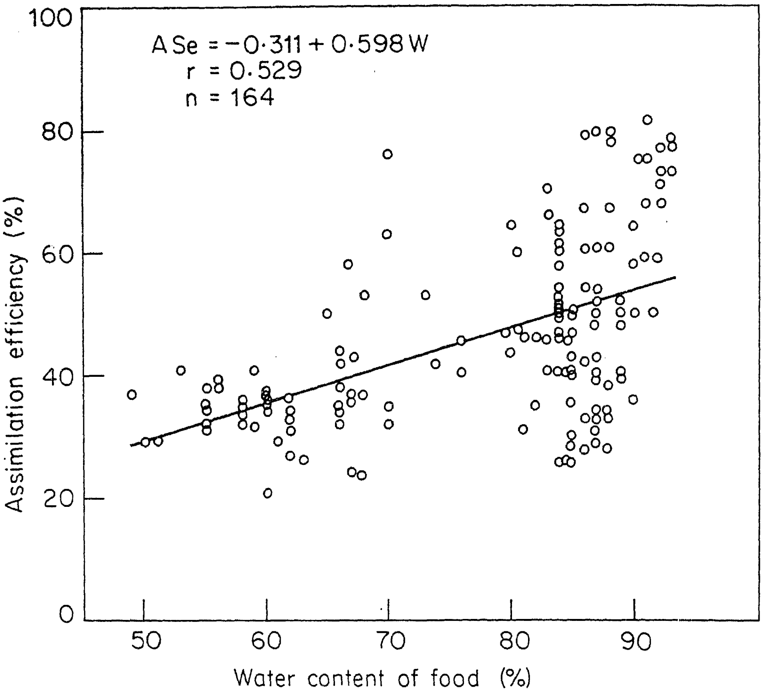

Figure 2. Assimilation efficiency of lepidopterans as a function of water content of food.

content. It is therefore suggested that there is a need for the collection of more information on this aspect before the observation of Scriber could be confirmed or contradicted.

The ability to digest and assimilate the plant parts by phytophagous insects may considerably be altered by the presence of digestibility reducing secondary chemicals such as tannin (e.g. Feeny 1969). Feeny (1970) observed that the Ase of tested lepidopterous larvae decreased with increasing tannin content; however, the data reported by him do not lend themselves to estimate the order of importance of leaf N, water and tannin in determining Ase. Incidentally, Coleopterans are known to display a high tolerance to tannin compared with lepidopterous larvae (Bernays 1978; Fox and Macauley 1977). Information on the importance of other digestive reducing substances (DRS) in determining the Ase of lepidopterans is vague. For instance Rausher (1981) fed *Battus philenor* larvae with young and old leaf of *Aristolochia reticulata* and *A. serpentaria* containing different levels of N and DRS. When values for equal N consumption from young and old leaves were considered, he could find no differences in the growth rate of *B. philenor*, which implies that Ase has remained equal. Rausher (1981) came to the tentative conclusion that N is more important. While due consideration for DRS as a second co-predictor to increase the precision of prediction of Ase (to increase the *r* over 0·9 as well as to decrease the SE to 7·7, as has been reported for fishes, reptiles, aquatic insects, amphibians, polychaetes; Pandian and Marian 1985b, c, d, e) is considered necessary, but it is too early to consider it in the present work. What seems to be more important is that it is not the N content of plant food, but the ultimate availability of N that determines Ase. To determine N availability of the plant, one needs to know the profile of DRS in host plants. While much information is still required about DRS, it may be noted that our predictive model can easily accommodate the components of DRS indicated below:

$$\text{Ase} = a + b\text{N} + b_2\text{W} + b_3\text{DRS}.$$

In an attempt to study the effect of different protein qualities on the Ase of *Bombyx mori*, Horie and Watanabe (1983b) fed the silkworm on a variety of synthetic diets containing different N levels. The recalculated values of Ase reported by them are presented against food N in figure 3, from which the following became apparent: (i) Irrespective of changes in protein quality, Ase shows a highly significant correlation ($r = 0{\cdot}9$) with food-N, and this finding confirms our concept of using food-N as a predictor of Ase. (ii) The levels of the slope describing the Ase-food-N relationship appear to be fixed by the protein quality. Thus, the trend obtained for milk casein is far higher than that of gluten. Briefly, N content of food determines the trend, but the protein quality determines the level of the trend. (iii) The cumulative trend obtained for Ase-food-N relationship of all the synthetic diets stands far lower than that reported for the lepidopterans fed on natural plants. It is not clear whether

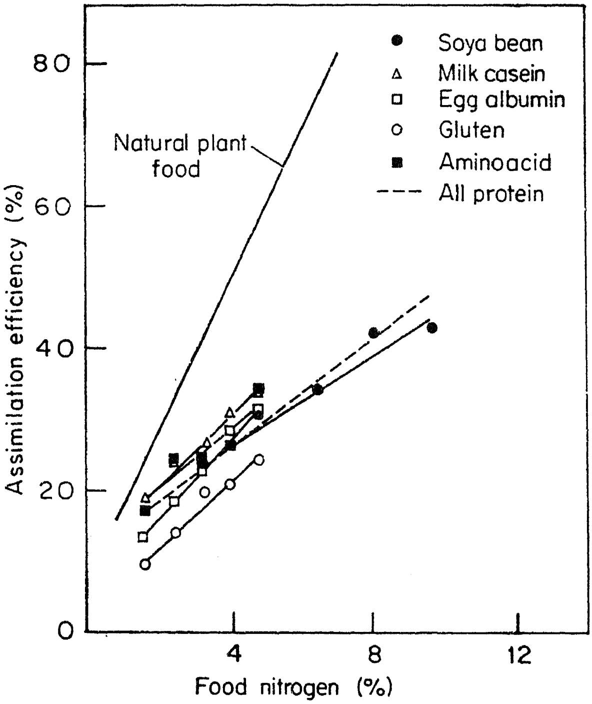

Figure 3. Assimilation efficiency of final instar larvae of *Bombyx mori* fed on synthetic diets containing different proteins as a function of food nitrogen. The Ase values reported by HORIE & WATANABE (1983b) were regressed and presented for comparison; the trend obtained for the relation between food nitrogen and Ase of lepidopterans fed on host plants (reported in figure 1 of this paper) is also given.

the low water content of the synthetic diet is responsible for lowering the slope obtained for the synthetic diet. In this context a report by Schramm (1972) is interesting; he observed that synthetic diet for insects generally must contain a minimum of 24% or more of protein but natural feeds containing protein level as low as 14% were adequate for supporting a moth and a beetle. Hence natural and synthetic diets appear to differ in their ability to get assimilated and to support insect growth. Hence, much is to be known to understand the significant differences in the Ase values obtained for natural and synthetic feeds; however it is important for the present to note that food N holds a significant positive relation with Ase.

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Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth)

M BALAKRISHNAN, V E SOKOLOV* and O A BASURMANOVA*
Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695 581, India
*Institute of Evolutionary Animal Morphology and Ecology, USSR Academy of Sciences, 33 Leninski Prospect, Moscow, USSR

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Abstract. The ultrastructure of the flank gland tissue of both sexes of the Indian musk shrew, *Suncus murinus viridescens* was investigated. The undifferentiated and differentiated sebaceous secretory cells in the glands of both sexes possess 1–8 mitochondrial dense osmophilic inclusions. A few inclusions of medium electron density, and clear vacuoles were also seen in the mitochondria. The muscle fibres in the glandular complex were devoid of these characteristic mitochondrial inclusions. Secretory activity of the well differentiated sebaceous cells was characterised by the presence of a number of large secretory granules. Mitochondria and polyribosomes were found in close association with the secretory granules. The flank gland tubules of the shrew were highly vascularised.

Keywords. Indian musk shrew; *Suncus murinus viridescens*; flank gland; sebaceous tubules; ultrastructure; mitochondrial inclusions.

1. Introduction

Specialized integumentary glands play an important role in the mammalian social behaviour. Ethological and histological parameters concerned with mammalian olfaction have been the focus of attention of most of the investigations on mammalian chemoreception during the past few years (Müller-Schwarze 1983; Balakrishnan and Alexander 1985). Information on the fine structure of mammalian sebaceous glands has also accumulated in the past few years (Kurosumi 1961; Ellis and Henrikson 1963; Sansone *et al* 1970; Bell 1974; Gorgas and Völkl 1984; Jenkinson *et al* 1985; Sokolov *et al* 1986).

Although both sebaceous and sudoriferous glands of mammals have odour producing properties, these glands are widely different in their structural organisation, chemical composition and in the functional process of secretory activities (Mykytowycz 1970; Quay 1972; Strauss *et al* 1976). The specialized integumentary glands in the Indian musk shrew, *Suncus murinus viridescens* have been the subject of detailed investigation in recent years (Balakrishnan 1975; Balakrishnan and Alexander 1985). An investigation on the fine structure of the sebaceous cells of the flank gland of the male shrew was also made (Balakrishnan *et al* 1984). The present report represents the results of further detailed investigations on the ultrastructure of the flank gland of *S. m. viridescens*.

2. Materials and methods

Adult shrews of both sexes were trapped live on the University Campus, Kariavattom, and were kept in individual wire-mesh cages for 2–3 days on a regular supply of minced beef sprinkled with shark liver oil and tap water *ad libitum.* The fur of the flank gland area was shaved off using a fine blade and the glandular skin from both sides was dissected out from 4 males and 3 females under ether anaesthesia. Thin slices of the glandular tissue were fixed in 2·5% glutaraldehyde in 0·1 M phosphate buffer (pH 7·2–7·3). The fixed tissues were washed in the buffer for 2 h and post fixed in 1% osmium tetroxide in 0·1 M phosphate buffer. The samples were then carried to the USSR in the same buffer and dehydrated using alcohol and acetone in the ascending grades and embedded in Epon 812. Ultra-thin and 1 µm sections were cut using glass knives in LKB Ultrotomes III and IV. The ultra-thin sections were mounted on formvar-coated grids. These sections were stained with uranyl acetate and lead citrate as detailed by Reynolds (1963) and examined under a JOEL JEM 100 C Electron Microscope at an accelerating voltage of 80 kV. 1 µm sections were stained with toluidine blue and observed under a light microscope for cellular localisation of the secretory tubules and their pattern of arrangement.

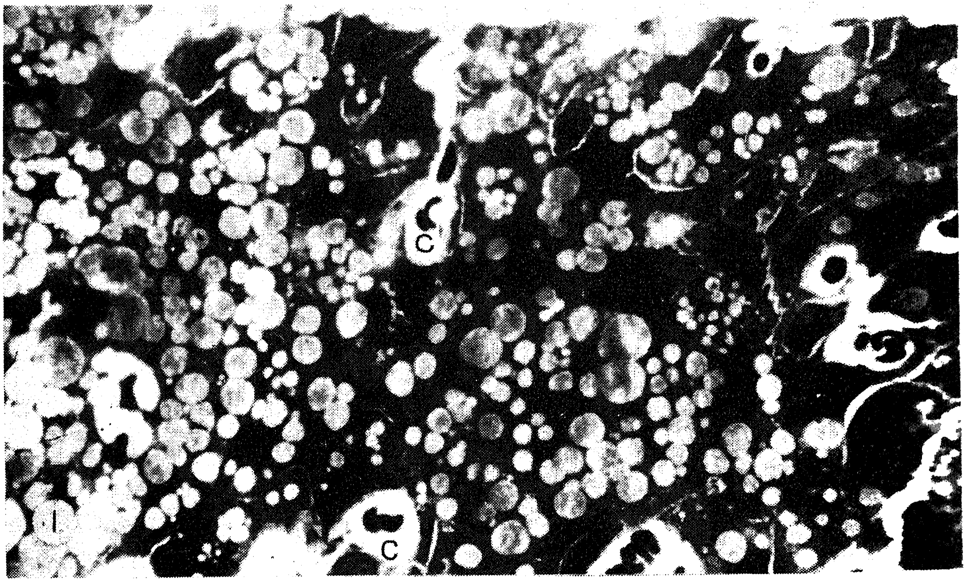
3. Results

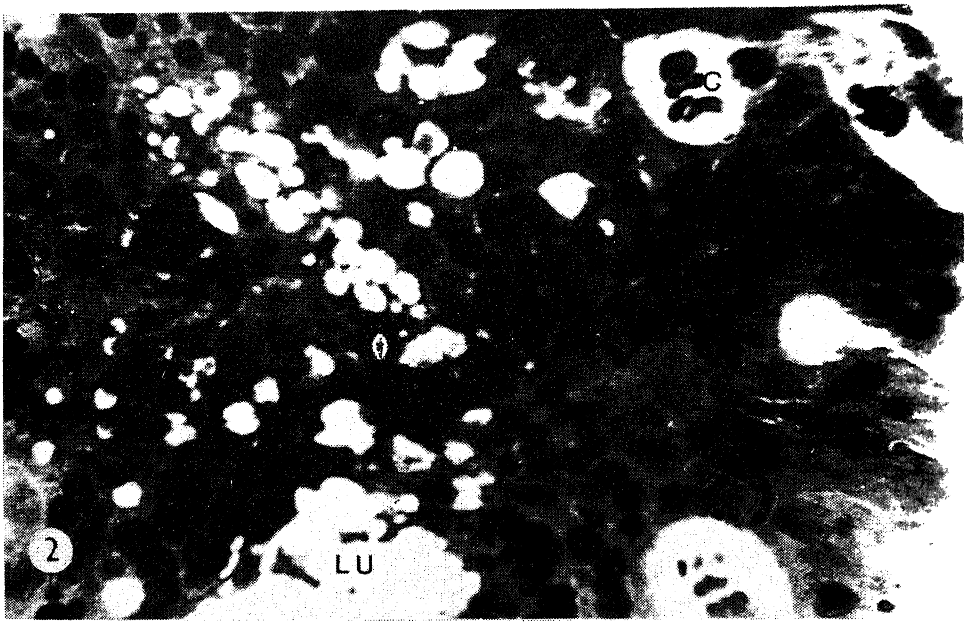
The flank gland tissue of *S. m. viridescens* is comprised of sebaceous and sudoriferous gland tubules. The latter are few in number and situated mainly on the glandular periphery. The undifferentiated sebaceous secretory cells were distinguished from the mature differentiated and differentiating cells by the absence of lipid secretory droplets in the former. The undifferentiated cells formed the basal layer of the secretory tubules whereas the inner lining of the tubular lumen was with well differentiated and disintegrating holocrine secretory cells. Disintegrating cellular structures were also seen in the sebaceous tubular lumen (figures 1 and 2). The differentiating and the differentiated sebaceous cells contained a few to several lipid droplets. Further, the differentiated cells were having comparatively larger granules.

One to eight electron dense osmophilic inclusions were observed in the mitochondria of the secretory cells. Mitochondria also contained a few inclusions of medium electron density. Some of the mitochondria had 2–4 clear vacuoles (figure 3). Detailed observations have revealed that these were not artefacts. The mitochondrial inclusions were seen both in the undifferentiated and differentiated sebaceous secretory cells of the flank gland of both sexes of the shrew. These characteristic mitochondrial inclusions were absent in the adjacent muscle fibres. The mitochondria were in close association with the lipid droplets (figure 4). Many free ribosomes were seen throughout the cytoplasm of the secretory cells, and polyribosomes were situated around the periphery of the lipid droplets (figure 5). A large number of capillaries were found around the secretory tubules of the flank gland showing the high vascularisation of the gland (figure 3). Further, the invagination of capillaries into the sebaceous secretory tubules was evident even in light microscopic observations (figures 1 and 2). Some of the sebaceous cells were having a number of finger-like peripheral protrusions at the level of capillaries (figure 6).

4. Discussion

The flank gland of the Indian musk shrew consists of well developed sebaceous and a few sudoriferous secretory tubules (Balakrishnan and Alexander 1977a). No significant





Figures 1–2. **1.** Light micrograph of part of a sebaceous secretory tubule of the flank gland of a male shrew showing undifferentiated and differentiated cells of several layers. Note the invagination of capillaries with erythrocytes at definite levels in the glandular tubule. Large secretory granules are seen accumulated towards the tubular lumen (× 1875). **2.** Light micrograph of part of a secretory tubule of the flank gland of a male shrew showing the undifferentiated epithelial cells at the tubular periphery and differentiated disintegrating cells towards the inner wall of the tubule. A few epithelial structures of the disintegrated cells are also seen in the lumen (× 1500). (C, Capillary; LU, lumen of a sebaceous tubule).

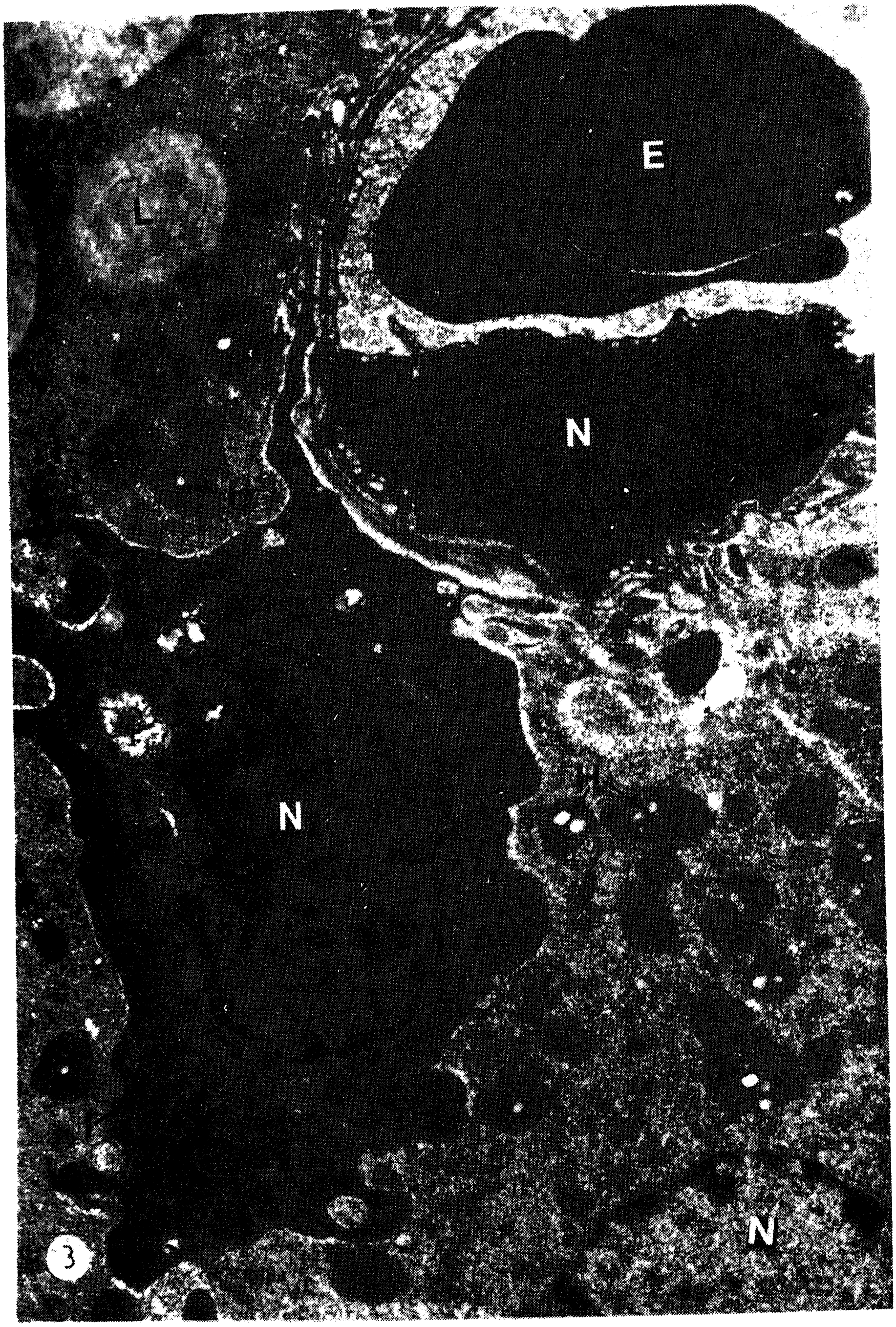


Figure 3. Part of the sebaceous tubule of the flank gland of a female shrew showing an undifferentiated cell on the left bottom and parts of differentiated secretory cells on upper left and on bottom right-hand sides. Note the mitochondrial dense inclusions (I) and vacuoles (holes, H) in both undifferentiated and differentiated cells. On the upper right-hand side, a capillary with erythrocytes (E) is seen (× 25,000) (N, nucleus; L, lipid droplet).

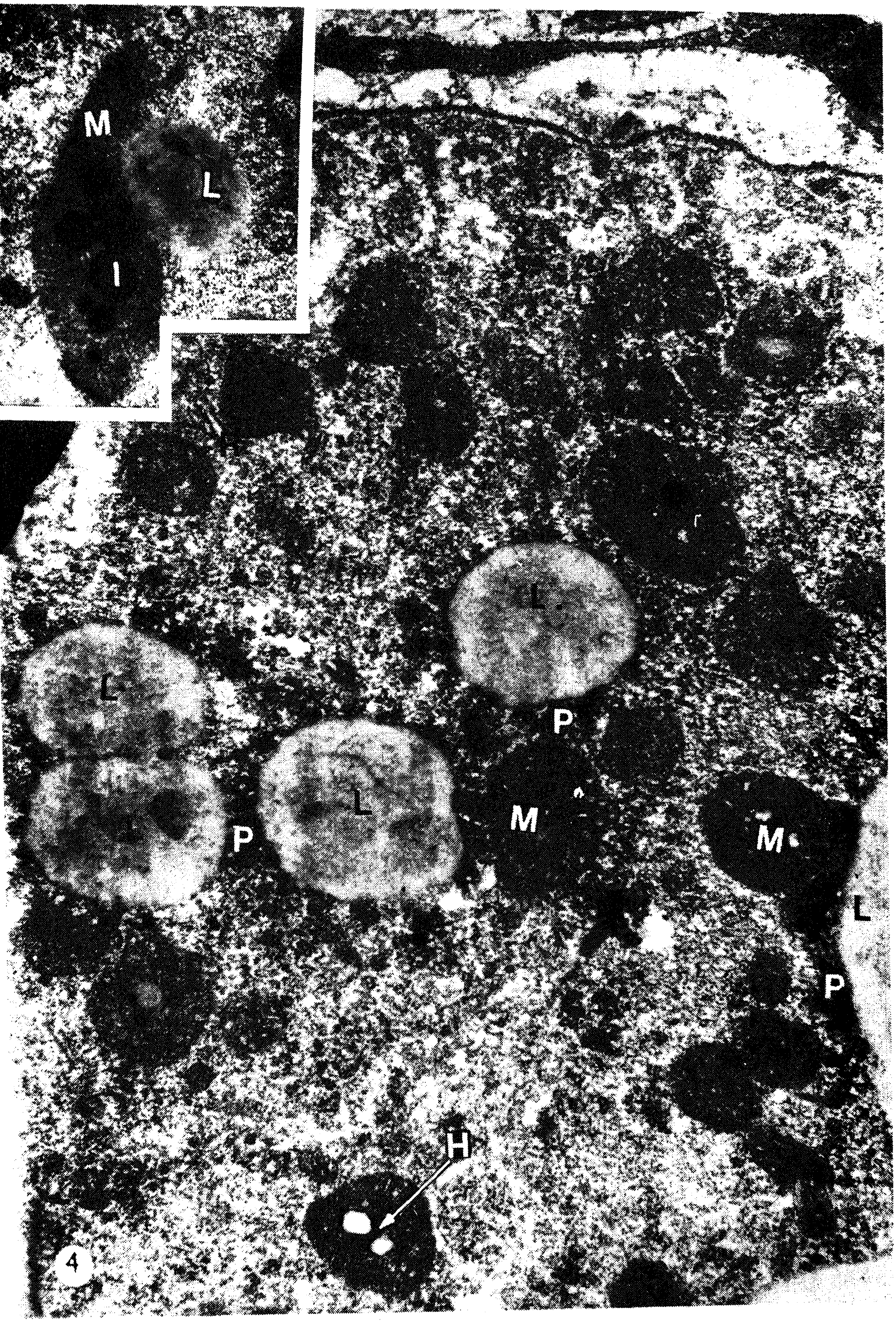


Figure 4.

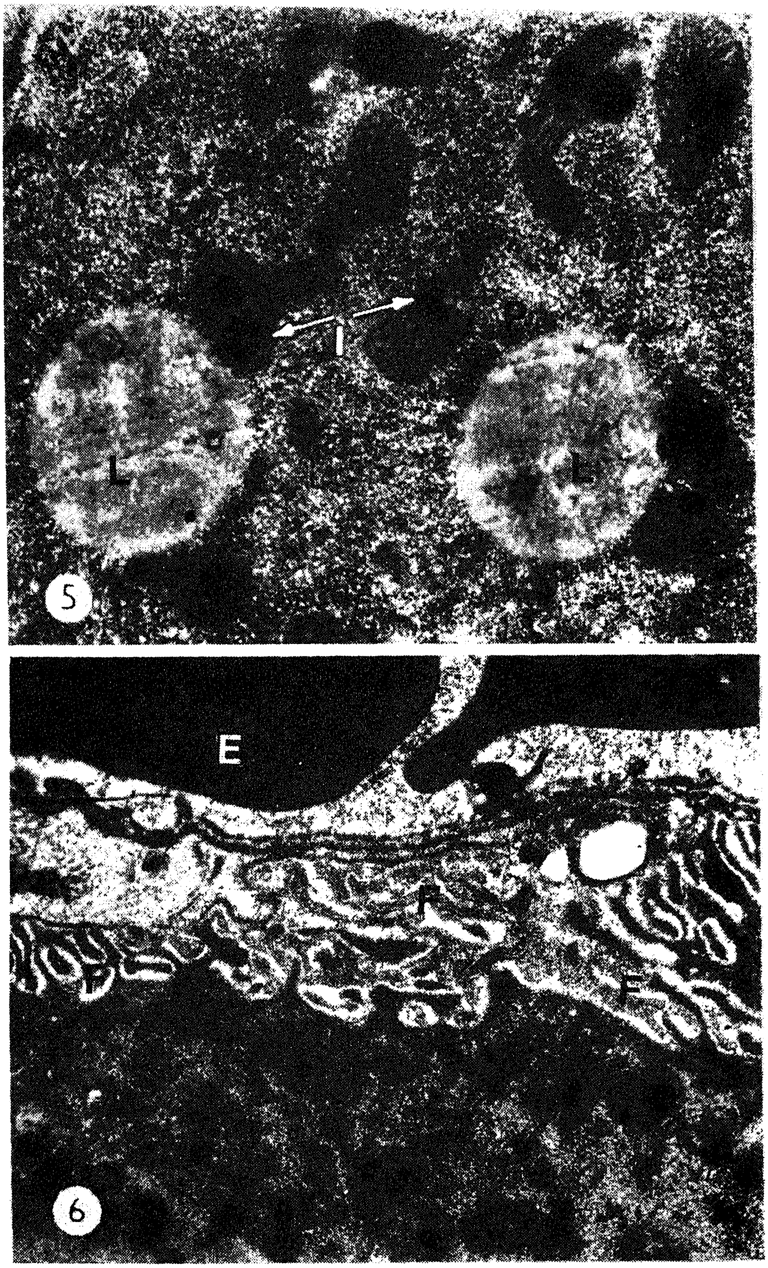
sexual dimorphism is discerned in the tubular pattern and in the structure of these secretory tubules. Lipid forms the major biochemical constituent of the flank gland sebaceous secretory material (Balakrishnan and Alexander 1977b).

Ultrastructural differentiation of the flank gland sebaceous secretory cells of both sexes of this species has now been elaborated. The undifferentiated sebaceous cells are characterised by the absence of any lipid droplets, whereas during the process of differentiation, small lipid droplets are developed and then fused to form large droplets as seen in well differentiated sebaceous gland cells. However, the presence of numerous mitochondria with characteristic electron dense inclusions (Balakrishnan *et al* 1984) in both undifferentiated and well differentiated cells of both sexes and their absence in the adjacent muscle fibres of this glandular tissue is of considerable interest. Presence of the mitochondrial inclusions thus forms a specific feature of the sebaceous glandular cells.

The size, number and nature of occurrence of the mitochondrial inclusions in the sebaceous gland tubules of male shrews have been discussed earlier (Balakrishnan *et al* 1984), and present observations revealed that the mitochondrial inclusions are similar in size, number and in the nature of occurrence in both sexes of the species. These results differ from the observations of Sansone *et al* (1970) on mice preputial gland only in the number of the mitochondrial inclusions. Balakrishnan *et al* (1984) found a few mitochondrial dense inclusions in the cytoplasm and in the ducts of the sebaceous tubules, probably as a result of their discharge from the mitochondria. The close association of mitochondria with lipid droplets in the differentiated gland cells suggest that the mitochondria not only have the function of energy production and release but also have a role in the lipid metabolism as suggested earlier (Bjersing 1967; Fawcett 1981; Jenkinson *et al* 1985). Fawcett (1981) also suggested that mitochondria involve in the metabolism of triglycerides and in the transport of some substrate to enzymes. The possible role of mitochondrial inclusions in the synthesis of the lipid droplets in specialized gland cells has already been suggested by Sansone *et al* (1970).

Observations of Sansone *et al* (1970) that the sebaceous cells of the preputial glands of immature mice also contained similar mitochondrial inclusions support the present findings on the presence of mitochondrial inclusions in the undifferentiated sebaceous cells of the flank gland of the shrew. The secretory activity of the flank gland of *S. m. viridescens* initiates when they are at about 9 days old (Balakrishnan and Alexander 1977b), far before their sexual maturity. This indicates the need for further ultrastructural investigations on the flank gland tissue of immature shrews, to confirm the probable role of the mitochondrial inclusions in the secretory function. The relationship between the electron dense inclusions, electron medium dense inclusions and the vacuoles are still unknown. However, we would suggest that the vacuoles in the mitochondria may be formed as a result of the metabolic activities of the electron dense inclusions or due to the discharge of these inclusions from the mitochondria.

Figure 4. Part of a differentiated sebaceous cell of the flank gland of a female shrew showing the lipid droplets, mitochondria (M), short cisternae of rough endoplasmic reticulum (R), many free ribosomes and polyribosomes (P) in the cytoplasm. Note the electron dense inclusions, medium dense inclusions and clear holes in the mitochondria (H) (× 25,000). A mitochondrion with electron dense inclusions is seen in the inset. The mitochondrion is situated in close contact with a lipid droplet (L) (× 30,000).



Figures 5–6. 5. Part of the cell of the flank gland of a female shrew showing the close association of mitochondria with lipid droplets. Polyribosomes are also found close to the periphery of the lipid droplets (L). Large number of free ribosomes are seen in the cytoplasm (× 25,000). **6.** Part of the sebaceous secretory tubule of the flank gland of a female shrew showing a capillary at the upper portion, below which is part of an undifferentiated gland cell with finger-like peripheral protrusions (F) (× 30,000).

The capillaries lying on the peripheral boundary of the sebaceous secretory tubules invaginate at definite levels in the tubule, facilitating better vascularisation of the sebaceous glandular cells to maintain a higher metabolic rate. Further, the peripheral finger-like protrusions of the secretory cells at the level of capillaries would provide a larger area of absorption of materials brought through the capillaries, which may also be required for the secretory activities of these cells. The polyribosomes found near the periphery of lipid droplets may contribute necessary enzymes for the synthesis and development of the lipid droplets, which ultimately form the glandular secretion of considerable behavioural relevance (Balakrishnan and Alexander 1980).

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Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae)

K P SHAHI and S S KRISHNA*
Entomology Laboratory, Department of Zoology, University of Gorakhpur, Gorakhpur 273 009, India

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Abstract. Male/female individuals of the red cotton bug, *Dysdercus koenigii* (Fabr.) in a given population, when provided equal mating opportunities, exhibit interesting variability in their mating trends with more coitions occurring between sexes comprising 3- and 5-day old adults. Mating between females ingesting cotton leaf and cotton seed-fed males is a futile sexual exercise in this bug's reproductive programme yielding no output of fertile eggs. However, in a vice versa situation, the mateds deposit viable eggs the proportion of which, following a second mating with seed-fed males, gets appreciably augmented in the subsequent egg batch.

Keywords. *Dysdercus koenigii*; mating trends; adult nutrition; egg output; egg viability.

1. Introduction

Investigations into the mating behaviour of the red cotton bug, *Dysdercus koenigii* (Fabr.), a major pest of cotton and okra (Crowe 1977), have so far been limited to an (i) understanding of its regulation by corpora allata activity (Sharma *et al* 1972; 1979), (ii) elucidation of a relationship between duration of copulation and oviposition (Shahi and Krishna 1979b) and, (iii) assessment of the ability of differently aged males and females or antennectomised males held in breeding pairs to perform this sexual act (Shahi and Krishna 1979a). Information on the role of adult nutrition in influencing the reproductive potential of this pest is chiefly derived from the accounts published by Saxena (1969) and Shahi and Krishna (1981). Against this background knowledge, it was considered desirable to ascertain quantitatively (i) preferences exercised by a male/female of known age to mate with a similarly or variably aged member of the opposite sex in triple-choice tests and, (ii) the physiological outcome of copulation between males and females held on different dietary regimes in the bug's egg output and egg viability. Our findings obtained in these areas of study are reported here.

2. Materials and methods

All individuals employed in the various tests performed here were always obtained from a laboratory stock culture of this pest (Shahi and Krishna 1979b). Examination into the mating behaviour of male or female bugs entailed the designing of two different kinds of experiments. In the first type, 3 variously marked (by India ink on forewings) 3-day old male or female adult individuals (choice of age based on the known fact that mating readily occurs in this species from the 4th day (Shahi and Krishna 1979b)), previously held singly within muslin-capped vials (3 cm diameter; 10 cm height) containing water-soaked cotton seeds renewed daily, were given simultaneously equal chance during a 6 h duration of a 24 h time cycle (Shahi and Krishna 1979b) for 3 successive days to copulate at a time with 1 of 5 different similarly aged adult bugs belonging to the opposite sex. Mating, when occurred between a pair amongst the introduced individuals, was allowed to proceed only for 5 min after which the sexes were carefully dismantled. An interval of 15 min was found adequate for the individuals that entered into sexual union to ignore the previous mating experience and to return to normal behaviour and was always maintained between the termination of copulation and the reintroduction of the 3 marked adults to another new member of the opposite sex for mating. Number of coitions performed by each of the 3 marked male/female individual, which gets in all 15 mating opportunities (5 per day) during the 3-day experimental period, was eventually determined.

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*To whom all correspondence should be addressed.

The second type of tests (again limited to 3 days) was also aimed at obtaining similar information on mating in these bugs and its layout was fundamentally similar to that of the first with the difference that 0-, 3- and 5-day old adults of either sex constituted an 'experimental trio' wherein each member's incidence of copulation (based on number of matings) with 5 separate 3- or 5-day old individuals of the opposite sex was noted. To investigate into the effect of disparity in the food ingested by copulating males and females on the insect's reproductive potential, one of the following dietary arrangements was provided during the imaginal life of a male and a female housed, since emergence and also following a stipulated mating schedule, inside a glass container (7 cm diameter; 9 cm height) in different compartments separated by a brass wire mesh partition (Shahi and Krishna 1979c):

(i) Fresh cotton leaves for males and water-soaked cotton seeds replenished daily for females.
(ii) Reversal of the situation described in (i).
(iii) Similar to (i) till the females laid the first batch of eggs after which leaf-fed males were replaced by those of identical age nourished since emergence on seeds. Females were allowed to have a second mating with such chaste, seed-ingested males.
(iv) Water-soaked cotton seeds renewed daily for both males and females.

Both the sexes comprising a pair were allowed to undergo coition (first or second) only for a duration of 6 h, the period which enabled the mated to lay appreciable number of viable eggs (Shahi and Krishna 1979b). A male and a female were given chances to copulate for the first time only on the 4th day (in other words, on completion of 3 days) of their lives for reasons already stated above. Water from a moist cotton swab was only provided to the adults when held in pairs to facilitate sexual union. Number of total and viable eggs laid by the mated females at the end of their 1st and 2nd oviposition cycles were recorded in these experiments.

All tests outlined here have been adequately replicated and data on mating, egg output and hatchability of eggs comprehensively presented.

3. Observations

Figures 1 and 2 schematically depict our observations on mating trends in *D. koenigii* males and females subjected to variously formulated experimental situations. Out of 15 equal opportunities provided for copulation for each of the 3

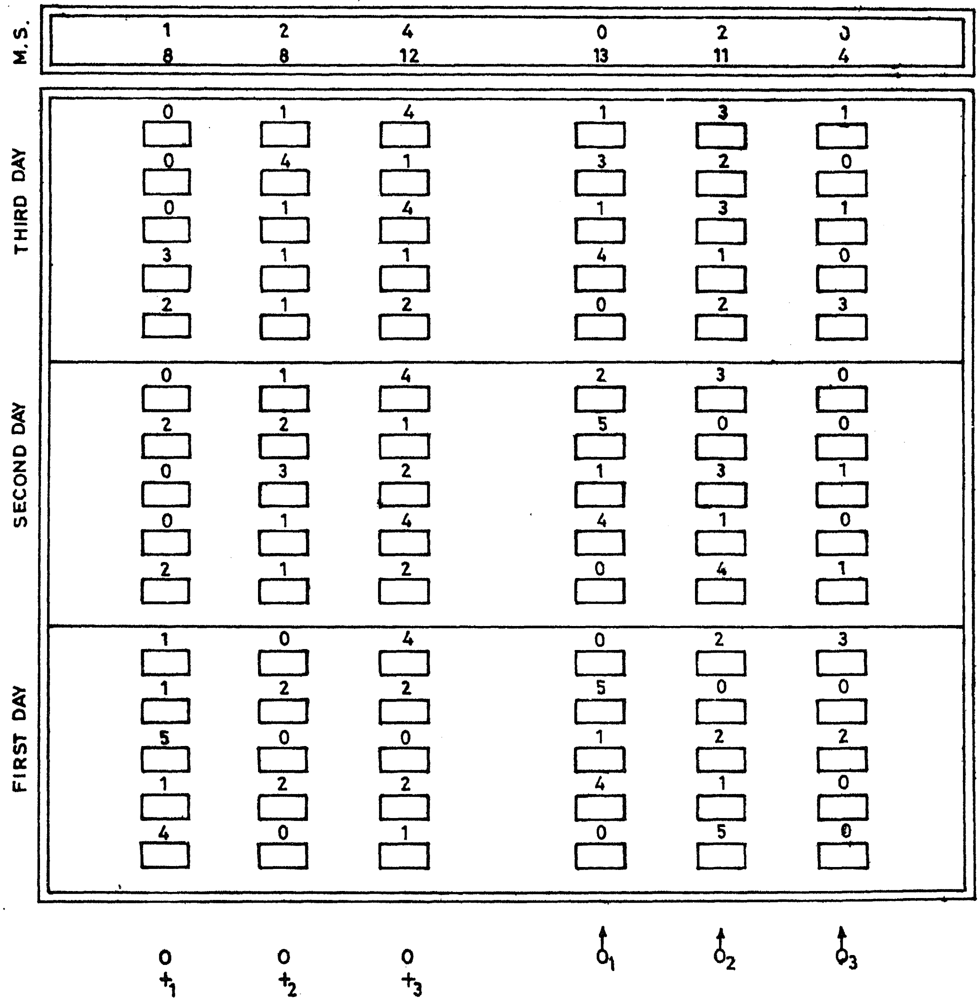

Figure 1. Schematic representation of the mating trends exhibited by 3 distinguishable males/females of *D. koenigii* given simultaneously equal opportunity to copulate with each of 5 different members of the opposite sex on 3 successive days.

Note: (i) Each set of 3 rectangular boxes placed at the same level within a segment above the experimental ♀$_{1,2\text{ and }3}$ or ♂$_{1,2\text{ and }3}$ constitutes a replicate, wherein quantitative estimate of their mating success with 5 separate 3-day old individuals of the opposite sex on each experimental day is given as absolute values above the appropriate box.

(ii) The topmost rectangular strip contains a break-up of the mating score (MS) data to indicate the minimum (upper entry) and maximum (lower entry) number of times a designated male/female could mate, when provided 15 copulation chances in all.

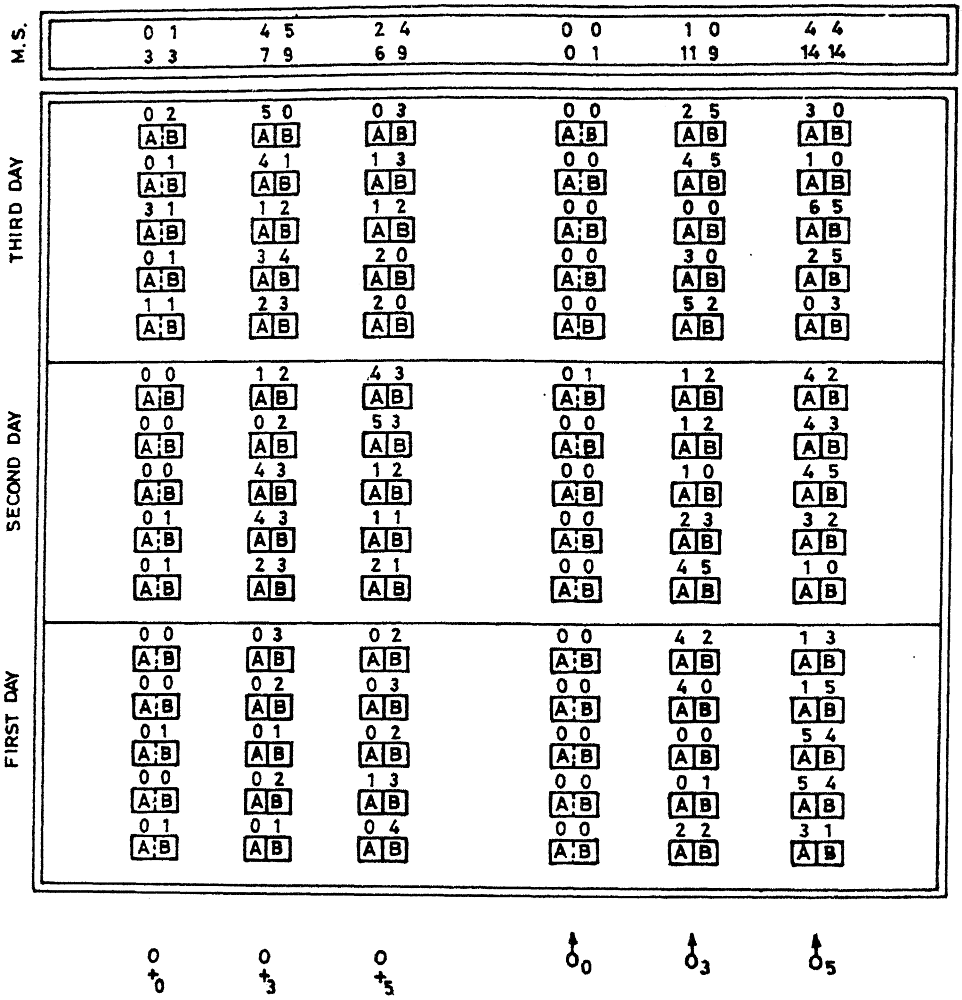

Figure 2. Schematic representation of the mating trends exhibited by variably aged (0, 3 and 5 days) and distinguishable males/females of *D. koenigii* given simultaneously equal opportunity to copulate with each of 3- or 5-day old 5 different members of the opposite sex on 3 successive days.

Note: (i) Each set of 3 rectangular boxes placed at the same level within a segment above the test ♀$_{0,3 \text{ and } 5}$ or ♂$_{0,3 \text{ and } 5}$ constitutes a replicate wherein quantitative estimate of their mating success with 5 separate 3-day old (segment A) or 5-day old (segment B) individuals of the opposite sex on each experimental day is given as absolute values above the appropriate segment of a box.

(ii) The topmost rectangular strip contains a break-up of the mating score (MS) data to indicate the minimum (upper entry) and maximum (lower entry) number of times a test male/female of a specified age could mate, when provided 15 copulation chances in all.

distinguishable males/females, all the tested females succeeded in utilizing at least one chance for mating with a male, the performance of this task in some females even going up to 80% (figure 1). This was not so, however, with the males in which 3 individuals (one of ♂$_1$ and 2 of ♂$_3$) never copulated with any female despite the maximal mating success (about 87%) in marked males exceeding that determined in counterpart females. In an identical number of opportunities presented to a group of 3 males or females of varying ages (0, 3 and 5 days) for mating with 3- or 5-day old bugs of the opposite sex, only the older (3 and 5 days) males and females in the test groups availed relatively larger proportion of these chances for coition (figure 2).

Females ingesting cotton seed diet from the time of their emergence, even when mated with leaf-fed males, were capable of depositing viable eggs, the percentage of which was more in the 1st than in the 2nd batch of total eggs laid by them. However, the egg hatchability level pronouncedly increased to a little over 95% in the second egg cluster laid by such seed-fed females following their mating with males who were also fed on seeds (figure 3). But females given access to feed only on cotton leaf, notwithstanding their entering into coition with males nourished on seeds, completely failed to oviposit.

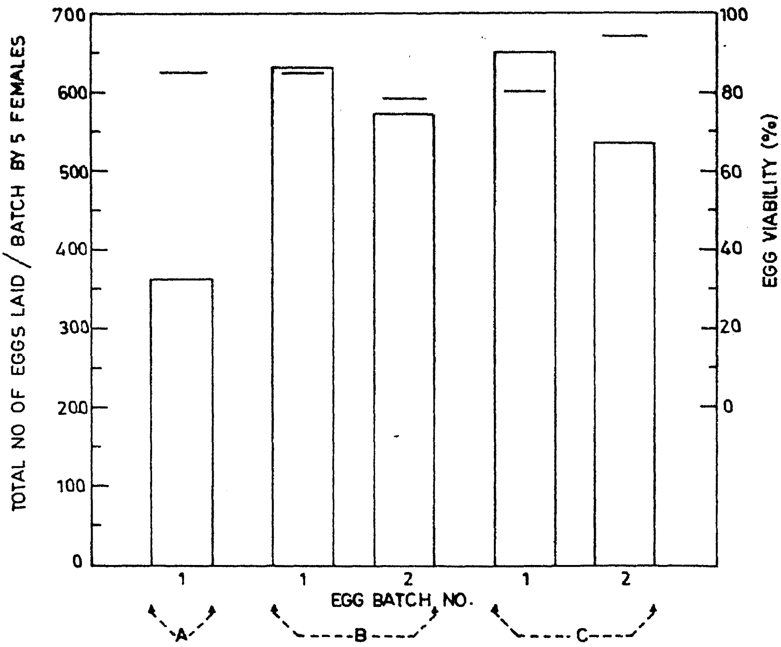

Figure 3. Histogrammic representation of total egg output and per cent egg viability recorded in the first and second batches of eggs laid by mated females of *D. koenigii* wherein the two sexes constituting a copulating pair were held on certain prescribed dietary regimens (data pooled from 5 females).

A. Males and females fed on cotton seed. **B.** Males fed on cotton leaf and females fed on cotton seed. **C.** Same as B till the females laid the first batch of eggs after which they were paired with cotton seed-fed males.

Note: Full height of each bar in the figure represents total number of eggs per batch by 5 females.

Level of horizontal line within or above a bar shows the value pertaining to the per cent viability of eggs laid in a batch by the females.

4. Discussion

Interesting variations were noticed in the mating characteristics of the red cotton bug despite the different tested individuals getting equal chances for copulation. While no forthright statements can be put forth at this stage to explain these phenomena, the findings obtained in this study offer the following possibilities:

(i) Some males and females of identical age in a sample population are genetically geared, behaviourally and physiologically, to exhibit their greater competency over others for winning the members of the opposite sex to mate more frequently.
(ii) The sexual urge and receptivity of males and females of mixed ages involved in mating get augmented by certain deep-seated, yet to be determined, age-related physiological and behavioural mechanisms operating during the reproductive life of this pest.

Changes effectuated in the nutrition of copulating adults markedly influenced the reproductive potential of this pyrrhocorid. The inability of cotton leaf-fed females to lay even a single egg, despite their mating with seed-ingesting males, unlike those females nourished on seeds and allowed to copulate with males fed on identical diet or on leaf, clearly indicates that nutrition from cotton seed is mandatory for the females of *D. koenigii* after their eclosion to initiate ovarian development and egg maturation (Saxena 1969) and to deposit fertile eggs following their insemination by such variably fed males. Presumably the relatively better protein constitution (quality- and quantity-wise) of the seed in contrast to that of the leaf (Shahi and Krishna 1980) favours the appropriate physiological processes connected with the production and release of viable eggs in females held on such experimental regimens. The occurrence of a marked elevation in the number of hatched eggs in the egg batch deposited by females (earlier mated with leaf-fed males) subsequent to copulation with seed-ingesting males hints at the likelihood of the sperms of the latter males being more virile and succeeding, as much as possible, in effectively displacing those of leaf-fed individuals, a feature associated with sperm competition phenomenon (Parker 1970), to fertilize maximum number of eggs following second mating. These findings unquestionably warrant a deeper probe into this thought-provoking issue to test the validity of this postulation.

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Occurrence of gastric absorption in the cichlid fish, *Sarotherodon mossambicus*

M NARAYANAN* and S V JOB
School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India
*Present address: Department of Zoology, St. Xavier's College, Palayamkottai 627 002, India

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Abstract. Absorption in the stomach region has been quantified in the freshwater fish, *Sarotherodon mossambicus*. When fish of different size groups were fed with wheat flour, irrespective of the size group, the maximum gastric absorption averaged to 26%. However, the time required to attain the maximum percentage of gastric absorption differs with size of the fish; in smaller individuals (3 g) it occurred within 90 min and in larger individuals (8 g) it took 60 min.

Keywords. Gastric absorption; feces; intestine.

1. Introduction

With reference to absorption of digested food, the old belief was that this took place in the digestive system below the stomach and not at all in the stomach. However, indications of gastric absorption are no longer an unknown fact as such evidence is now forthcoming and not confined to fish alone (Slyke and White 1911; Greene 1913; Dawes 1930; Karel 1948). Though these workers record the incidence of fat or protein absorption in the stomachs, quantification of gastric absorption in terms of energy absorbed is entirely wanting. Therefore, an attempt was made to study the gastric absorption in two size groups of *Sarotherodon mossambicus* fed with an artificial food.

2. Materials and methods

Two size groups (A = 3 g; B = 8 g) were collected from ponds in and around Madurai. They were acclimated to the laboratory conditions of 14 h photoperiod and 28 ± 2°C for a period of 10 days and during those periods they were fed *ad libitum* with the safranin mixed wheat flour. Safranin was added mainly to give colour so that they can be easily located in the different regions of the gut.

Fifteen fish from each size group were kept separately in the aquarium and were fed. Samples of 3 fish were removed from each size group every 30 min after feeding till the first coloured feces were released in the aquarium. The fish removed for study of the gut contents were stunned immediately and were cut open and the contents of stomach and adjoining 10 cm of intestine were carefully removed and separately oven-dried. The samples were analysed for calorific content using the Oxygen Bomb calorimeter.

3. Results

The feces of fish of size groups A and B appeared at 110 and 115 min respectively after consuming the food and the observations were continued up to 2 h.

In group A, the stomach contents of fish showed a decrease in the energy value from 22·56 ± 0·887 to 16·38 ± 0·858 KJ/g of stomach contents within 90 min after the food intake. Two hours after consumption, the stomach contents showed 18·25 ± 0·929 KJ/g. The energy value of the intestinal contents also showed an initial decrease from 22·45 ± 1·013 to 14·26 ± 0·767 KJ/g and an increase to 16·34 ± 0·699 KJ/g thereafter (table 1).

In groups B, however, the energy value of the stomach contents declined from 22·56 ± 0·887 to 16·89 ± 0·703 KJ/g within 60 min and the energy values at 90 and 120 min were 19·00 ± 0·941 and 16·87 ± 0·979 KJ/g respectively. The energy value of intestinal contents dropped from 20·92 ± 0·858 KJ/g after 30 min of feeding to 15·17 ± 0·481 KJ/g after 90 min and then increased to 16·70 ± 0·837 KJ/g after 120 min (table 1).

4. Discussion

In the literature, there is evidence for the gastric absorption of fat (Greene 1913; Dawes 1930; Blake 1930; Al-Hussaini 1949). However, the information about the absorption of protein in the stomach is controversial. The work of Slyke and White (1911) stands rather alone in the literature as a noteworthy attempt to study by biochemical means the digestion and absorption of protein in fish. They fed chopped boiled beef to dogfish and found that 25% of the food was absorbed in the stomach. Soulima (1919), however, found no evidence for protein absorption in the stomach of *Scyllium catulus*.

In the present experiment, when the fish were fed with wheat flour about 26% of the energy was absorbed within 90 min while the food was in the stomach region,

Table 1. Gastric absorption in *S. mossambicus* (groups A and B) fed with wheat flour. Each value represents mean (± SD) of at least 3 replications.

Size group	Time (min)	Energy content in Stomach (KJ/g)	Energy content in Intestine (KJ/g)	Absorbed in stomach (%)
	0	22·56 ± 0·887	—	—
	30	21·00 ± 0·360	22·45 ± 1·013	6·9
A	60	17·38 ± 0·582	15·54 ± 0·808	23·0
	90	16·38 ± 0·858	14·26 ± 0·767	27·4
	120	18·25 ± 0·429	16·34 ± 0·699	19·0
	0	22·56 ± 0·887	—	—
	30	18·41 ± 0·657	20·92 ± 0·858	18·4
B	60	16·89 ± 0·703	17·22 ± 1·205	25·1
	90	19·00 ± 0·941	15·17 ± 0·481	15·8
	120	16·87 ± 0·979	16·70 ± 0·837	25·2

irrespective of the size of the fish. It is seen further from table 1 that the maximum percentage of absorption of energy (27·4%) occurs 90 min after consumption in group A and after 60 min (25·1%) in group B indicating that gastric retention of food (Narayanan 1980) appears to be linked with this peak absorption level. Further in group A, the caloric value of food decreases from 22·56 KJ/g to 16·38 KJ/g up to this point and from 22·56 KJ/g to 16·89 KJ/g in group B. But in both cases after this point there is once again an increase in the caloric value of the stomach contents indicating clearly the consumption of a fresh meal.

So, by mixing the already partially digested and fresh food, the energy content of the stomach had gone up. In group A, however, the second decrease in the energy content of the stomach was on account of coprophagy because after autopsy it was discovered that the stomach contained considerable amount of fecal material which naturally with their lower calorific content would bring down the calorific value of the stomach contents.

However, when the energy values of food in the stomach and intestine were compared, there was some kind of anomaly in that the energy value of food in the intestine immediately following the stomach was slightly higher than that of the stomach (table 1). This could be due to the fact that since food consumption is somewhat continuous as the food fills in the stomach, it is passed to the intestine before being fully digested and then only partially absorbed in order to make way for more food to come into the stomach. A similar condition was indeed observed in the fish, *Rhinomugil corsula*, where in the same animal the energy value of intestinal contents was more than that of the stomach contents when represented as KJ/unit weight of the contents (S V Job, unpublished results). Moriarty and Moriarty (1973a, b) also reported a similar occurrence in *Tilapia* and *Haplochromis* fed on plankton. They observed that only a part of the ingested plant material remained in the stomach and much of the meal passed straight to the intestine and they ascribed the poor assimilation to this phenomenon.

Thus it is clear from this work that in *S. mossambicus* there is considerable absorption of energy in the stomach itself. As far as the time course of absorption is concerned, there is rapid absorption in the first 60 min from the time food enters the stomach and the fish goes in for more meal and the cycle is repeated. Earlier workers did not follow the time course of food in the gut and especially of gastric absorption and this work is the first record. Weinland (1901) recorded retention of food in the stomach of selachians for 30 days or more and in one case for 18 days and Dobreff (1927) noted the first defecation in dogfish 5 days after a meal. Of course, the anatomy of the selachian stomach with its spiral valve indicated that the food must be in the stomach for the purpose of digestion and absorption for a considerably longer time than in cases such as *S. mossambicus* in which the stomach is a mere bag. Still about 27% of absorption of food energy was accounted for in the stomach region in this fish, *S. mossambicus*.

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Effect of an insect growth regulator on the growth and development of the tobacco caterpillar, *Spodoptera litura* Fabricius

G T GUJAR and K N MEHROTRA
Division of Entomology, Indian Agricultural Research Institute, New Delhi 110 012, India

MS received 31 March 1986

Abstract. Effect of an insect growth regulator BASF LAB 153 959 I was studied on the various stages of *Spodoptera litura* Fabr. BASF growth regulator of substituted urea type had a similar action as diflubenzuron and was effective against larval stages of *Spodoptera litura*. It was ineffective against egg and pupal stages due to poor contact action. It affected adversely fecundity of female moth and fertility of eggs.

Keywords. Insect growth regulator; BASF LAB 153 959 I; *Spodoptera litura*.

1. Introduction

Analogues of the benzoyl phenyl urea have a great potential in selective control of insect pests (Wellinga *et al* 1973a, b). Although the exact mode of action of these chemicals is not known, they affect the chitin synthesis, thereby inhibiting the moulting process (van Daalen *et al* 1972). Diflubenzuron (4–(4–chlorophenyl)–amino carboryl-2, 6–difluorobenzamide) was the first such compound tried extensively on a field scale against a number of insect pests.

Recently, a new insect growth regulator, BASF LAB 153 959 I, a substituted phenyl urea, has been developed as an insecticide with high activity against lepidopterous and coleopterous insect pests. This communication reports the results of its effects on growth and development of the various stages of the tobacco caterpillar, *Spodoptera litura* Fabr. in the laboratory.

2. Materials and methods

Insects used in the present study were from various stages of *S. litura* obtained from a laboratory colony maintained at 27° ± 2° C and 60–80% RH on the leaves of castor, *Ricinus communis* Linn.

BASF LAB 153 959 I (BASF growth regulator) is an experimental insecticide of BASF Aktiengesellschaft, Ludwigshafen, FRG. It was obtained as 50% suspension concentrate.

The ovicidal activity was tested as follows: Eggs 20 in number, 0–12 h old were placed in petridish (51 cm diameter), pretreated with 0·5 ml of various concentrations viz $2 \times 10^{-3}\%$, $2 \times 10^{-2}\%$, $2 \times 10^{-1}\%$ and 2%. For each concentration there were two replications. Observations on egg mortality were recorded 3 days after treatment based on egg-hatchability.

The effect of BASF growth regulator on larval-larval transformation was studied on 2nd instar (3 day old) larvae. Each castor leaf was sprayed with 1 ml of various concentrations of the compound. 20 equal size larvae were fed on the treated leaves for 36 h with proper control. Insects were fed fresh, untreated leaves after 36 h. Mortality in various treatments was recorded after a 4 day period. In another experiment, 5th instar, 7 day old larvae were fed *ad libitum* castor leaves treated with various concentrations for two days to study the effect of BASF growth regulator on last larval-larval transformation and subsequent growth and development until adult emergence.

The effect of BASF growth regulator on larval-pupal transformation was studied by feeding treated leaves to the last instar larvae of various ages for a 12 h period. Larvae were then fed on fresh, untreated castor leaves until pupation. Observations on mortality and growth deformities were recorded 4 days after pupation in control.

The effect of BASF growth regulator on pupal-adult transformation was studied by directly spraying 1 ml of various concentrations on 2 day old pupae. Observations on mortality and abnormalities in adult moths were recorded as soon as they emerged.

The chemosterilant effect of BASF growth regulator was studied by injecting 2 μg of the compound in 16 h old female moths. The treated females were paired with normal males. Observations were recorded on the fecundity and egg-hatchability.

3. Results and discussion

3.1 *Ovicidal effect*

BASF growth regulator did not show any ovicidal effect although diflubenzuron has been reported to possess ovicidal activity (Ascher and Nemny 1974; Grosscurt 1978; Natesan and Balasubramaniam 1981). The direct contact activity to eggs is known to be species dependent and is influenced by many factors (Grosscurt 1978).

3.2 *Effect on larval-larval transformation*

BASF growth regulator affected successful transformation of 2nd instar larvae into 3rd instar larvae when fed with leaves treated with various concentrations of the compound. It was seen that at concentration of $2 \times 10^{-3}\%$, 95% of 2nd instar larvae could not successfully moult to 3rd instar. These larvae had partially ruptured head capsule with untanned head inside, thus giving an appearance of 'swollen head'. At the concentration lower than $2 \times 10^{-3}\%$, larvae did undergo normal moulting but their subsequent growth was slower when compared to control. Larvae feeding on leaves treated with higher concentrations such as $2 \times 10^{-2}\%$ and $2 \times 10^{-1}\%$ died at the time of moulting without showing any morphological aberrations except turning black in colour. In case of 5th instar larvae treated with BASF growth regulator, 100% mortality was observed at last larval-larval transformation, that is at the end of 2 days feeding of treated leaves of castor in two treatments viz $2 \times 10^{-2}\%$ and $2 \times 10^{-3}\%$ (figure 1). These treatments caused severe loss of body fluid from larvae at the time of their death. At a concentration of $2 \times 10^{-4}\%$ high mortality of larvae was observed after a delay of 1 day. The larval-pupal transformation was also affected leading to the formation of 10% larval-pupal intermediates (L-P intermediates). At lower concentrations ($2 \times 10^{-5}\%$ and $2 \times 10^{-6}\%$) a significant number of dead pupae were observed suggesting that sublethal concentrations also possessed latent deleterious effects on growth and development of *S. litura*. These observations on

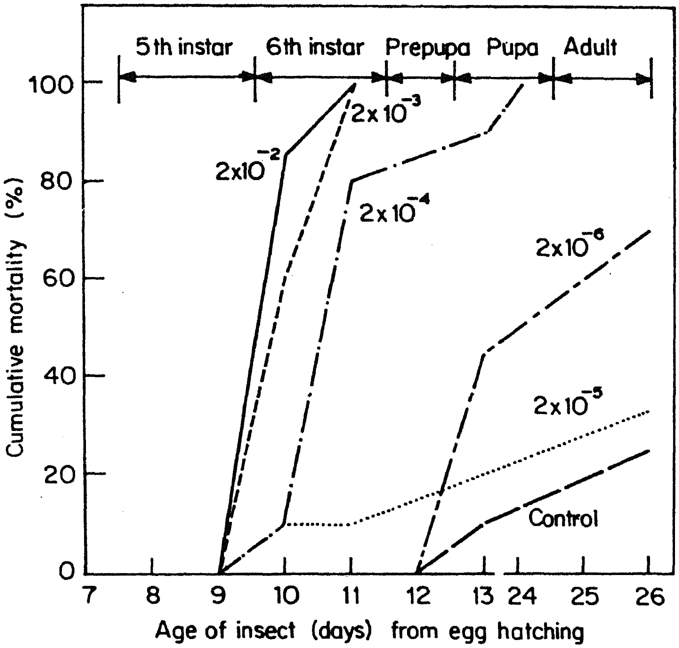

Figure 1. Effect of feeding of BASF growth regulator to 5th instar on subsequent growth and development of *Spodoptera litura* Fabr.

slower rate of growth of larvae when treated with sublethal doses are similar to those reported with diflubenzuron treatment by Sundaramurthy (1977) and Sundaramurthy and Balasubramaniam (1978) in *S. litura* and Salama *et al* (1976) in *Boarmia bistortata.*

3.3 *Effect on larval-pupal transformation*

The effect of this compound on larval-pupal transformation shows that at $2\times10^{-3}\%$, all last instar younger larvae moulted to L-P intermediates (table 1). However, 36 h old last instar larvae showed diminished response to produce L-P intermediates at the above concentration. These L-P intermediates were having distinct larval heads and varying degree of pupal integument all over body. Similar L-P intermediates have also been reported as a result of diflubenzuron treatment in *S. litura* (Sundaramurthy 1977) and *Spodoptera mauritia* (Beevi and Dale 1980) and appear to be characteristic effect of chitin synthesis inhibitors. Age-dependent response of *S. litura* to BASF growth regulator in producing juvenilizing effect was remarkably similar to the effect observed on juvenile hormone treatment (Srivastava and Prasad 1980). Treatment with high concentrations of the compound ($2\times10^{-2}\%$ and $2\times10^{-1}\%$) led to the failure of moulting in majority of treated insects with very high degree of blackening. These results show that BASF growth regulator was nearly 3–10 times more effective than diflubenzuron which was found to inhibit moulting to the extent of about 50% in *S. litura* at 0·0075–0·02% depending upon the various factors (Sundaramurthy 1977; Swamy and Punnaiah 1983).

Table 1. Effect of BASF LAB 153 959 I on the larval-pupal transformation of *S. litura*

Treatment (%)	Normal pupae (%)	L-P intermediates (%)	Larvae (%)
	Larvae 12 h old after penultimate moult		
2×10^{-1}	—	10	−(90[a])
2×10^{-2}	—	40	−(60[a])
2×10^{-3}	—	100	—
2×10^{-4}	90	—	10
Control	90	—	10
	Larvae 30 h old after penultimate moult		
2×10^{-1}	—	—	−(90[a],10[c])
2×10^{-2}	—	10	−(80[b],10[c])
2×10^{-3}	—	60	−(30[b],10[c])
2×10^{-4}	50	10	30(10[b])
Control	90	—	−(10[c])

Last instar larvae are formed 10 days after egg hatching.

[a] Black flacid loose body with prepupal shape, dead larvae under soil.

[b] Black alive larvae as above with 1 or 2 segment of abdomen partially tanned with pupal skin.

[c] Larvae black loose, as above, dead above soil.

Table 2. Effect of BASF LAB 153 959 I on pupal-adult transformation of *S. litura*.

Treatment (%)	Normal adults (%)	*Abnormal adults (with score) (%)	Dead pupae (%)
2×10^{-1}	10	80(2·56)	10
2×10^{-2}	50	50(2·40)	0
2×10^{-3}	60	35(1·43)	5
2×10^{-4}	65	25(1·40)	10
2×10^{-5}	90	10(1·50)	0
Control	95	5(1·0)	0

*Abnormal adults were scored over 4-point scale.

(1) Slightly twisted fore wings but adult normal.
(2) Moderately twisted fore- and hind wings but adult normal.
(3) Highly twisted shrunken wings exposing above 25% abdomen.
(4) Extremely twisted shrunken wings exposing about 50% abdomen but for size and shape adults normal. Score was calculated as below:

$$\text{Mean score} = \frac{(\text{Degree of abnormality} \times \text{No. of abnormal insects})}{\text{No. of abnormal insects}}.$$

3.4 *Effect on pupal-adult transformation*

The data on the effect of BASF growth regulator on pupal-adult transformation show that nearly 50% abnormal adults were formed at 2×10^{-2}% concentration with relatively high degree of abnormality (table 2). The mean score of abnormality in

Table 3. Effect of BASF LAB 153 959 I on longevity, fecundity and egg-hatchability of *S. litura*.

	Longevity (days)			
	Male	Female	Eggs/female	Egg-hatchability (%)
Control	8·4	7·4	1504 ± 764	68
Treatment (2 μg/♀)	7·8	6·2	1037 ± 450	6

adult moth was found to be positively correlated with the concentration. The abnormal adults were relatively weak and short lived. Similar morphological deformities to a greater extent have also been reported with diflubenzuron probably due to its high contact action. BASF growth regulator even at highest concentration of $2 \times 10^{-1}\%$ did not show any mortality of pupae suggesting that it has a poor contact action and is not effective as diflubenzuron on the pupal stage (Natesan and Balasubramaniam 1980; Swamy and Punnaiah 1983).

3.5 *Effect on fecundity of the female moth and egg-hatchability*

The data on the effect of BASF growth regulator on fecundity and egg-hatchability are presented in table 3. The treated female moth laid about 1037 ± 450 eggs with only 6% egg-hatchability, while in control female moth laid as many as 1504 ± 764 eggs with 68% egg-hatchability. Treatment of BASF growth regulator did not significantly affect the longevity of the female moths. Similar effect on egg-hatchability have also been reported with penfluron (Chattoraj and Dwivedi 1980).

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Heliothis species and their natural enemies, with their potential for biological control

C S PAWAR, V S BHATNAGAR* and D R JADHAV
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India
*Present address: FAO/CILSS IPM Project, BP 281, Kaolack, Senegal

MS received 28 April 1986

Abstract. Four egg and 24 larval parasitoids including one mermithid species have been identified from *Heliothis armigera* collected at and around ICRISAT Center. Twenty one insect and five spider species have been recorded as predators of *Heliothis*. The degree of parasitism varies according to the crop. Egg parasitism is absent on chickpea, and almost negligible on pigeonpea (0·3%). Most early larval parasitism occurs on pearl millet (50·7%), sorghum (49·5%), and chickpea (31·4%), whereas late larval parasitism occurs on pigeonpea (16·4%), and groundnut (11·5%).

The egg parasitoids, mostly *Trichogramma chilonis* Ishii, and the parasitoids of small larvae, mostly *Campoletis chlorideae* Uchida, are the most abundant natural enemies of *Heliothis* in the study area.

Keywords. *Heliothis* spp.; parasitoids; predators; pathogens.

1. Introduction

The International Workshop on *Heliothis* Management in 1981 held at the ICRISAT, reviewed *Heliothis* work and discussed the future research strategies to combat the ever increasing menace of *Heliothis* in national and international agriculture (ICRISAT 1982). It was recognised that work is required on a regional basis to develop integrated pest management programs for *Heliothis*. At ICRISAT Center, some components of integrated pest management, particularly of *H. armigera*, are under investigation. This paper reviews the results of 11 years of monitoring of *Heliothis* and the natural enemies of this genus.

2. *Heliothis* species at ICRISAT Center and its environs

Three *Heliothis* species—*H. armigera* (Hubner), *H. peltigera* (Schiff.) and *H. assulta* Guenee damage crops in India. The most important of these is *H. armigera* (Jayaraj 1981; Jadhav *et al* 1985). This is confirmed by 11 years light trapping data from ICRISAT Center in which *H. armigera* formed 99·2% of the catch, followed by *H. assulta* (0·6%) and *H. peltigera* (0·2%).

3. Host plants and seasonal population of *H. armigera*

Of the 96 cultivated and 61 uncultivated plant species that have been reported to be hosts of *H. armigera* in the Indian literature, 50 cultivated and 48 uncultivated

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species have been recorded at and around ICRISAT Center (Bhatnagar and Davies 1978). This wide host range covers most crops, including the ICRISAT's mandate crop: sorghum, pearl millet, groundnut, pigeonpea and chickpea.

The trend of larval population of *H. armigera* on ICRISAT crops is shown against the ICRISAT cropping schedule in figure 1. ICRISAT crops provide food for *H. armigera* from July until April, when there is a closed season of 2 months (May–June). During the closed season *H. armigera* survives largely on weeds. Thus, *H. armigera* can breed throughout the year at and around ICRISAT Center (Pawar *et al* 1984).

H. armigera feeds on the foliage and flowers of groundnut; the earheads of sorghum and pearl millet; the flowers and pods of pigeonpea; and the foliage, flowers and pods of chickpea. *H. armigera*, multiplying on rainy season crops, appears to exert high population pressure on postrainy season crops, principally pigeonpea and

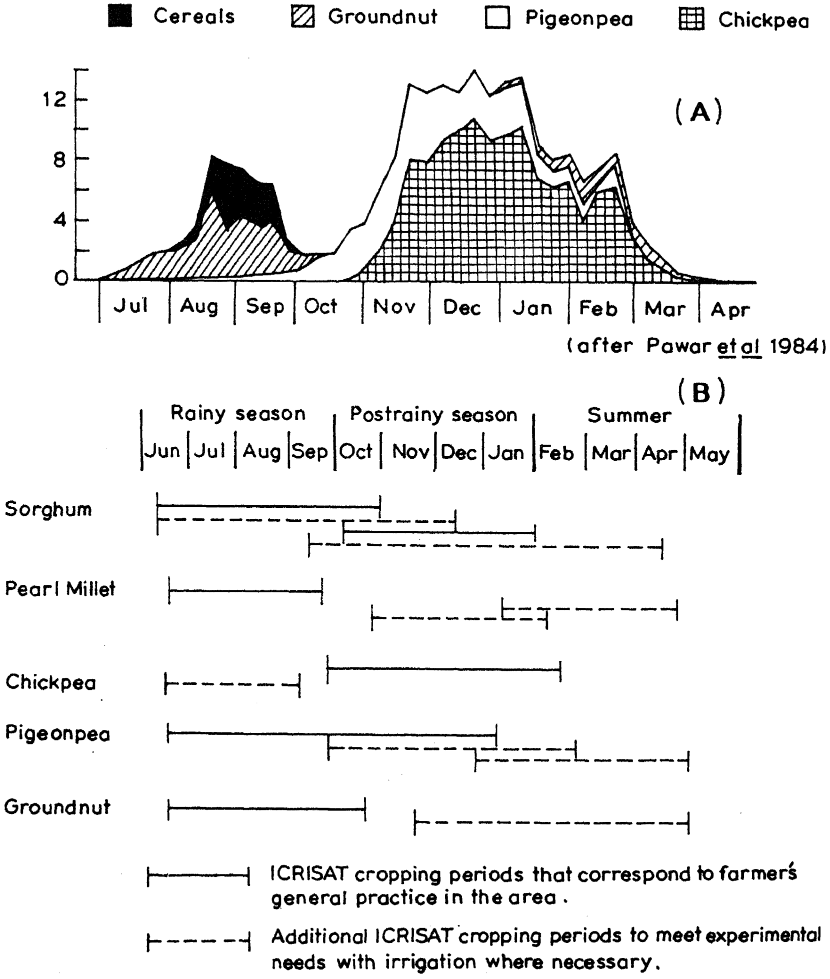


Figure 1. A. Trend of populations of *H. armigera* larvae on crops in the pesticide-treated areas of ICRISAT Centre, between 1979–80—1982–83. **B.** ICRISAT Centre's cropping schedule.

chickpea. The role of long distance migration within India and Africa in determining levels of infestation by *H. armigera* on different crops is now being investigated.

4. Natural enemies of *Heliothis* spp.

4.1 *Parasitoids*

The egg and larval parasitoids and their effect on *Heliothis* populations have been studied in relation to many plant species. Four egg and 24 larval parasitoids including one mermithid species have been recovered from over 80,000 eggs and 200,000 larvae of *H. armigera* collected from the ICRISAT Center farm and from farmers' fields (table 1). The parasitoids also recorded from *H. peltigera* and *H. assulta* are included in the table.

Of the egg parasitoids, *Trichogramma chilonis* Ishii is the most common. Hymenoptera and Diptera have been recovered from the larvae. Most Hymenoptera emerge from 1–3 instar larvae and from collections on cereals, whereas most Diptera emerge from 4–6 instar larvae from legumes. Among the Hymenoptera *Campoletis chlorideae* Uchida, and among the Diptera *Carcelia illota* Curran are most important; they occur on many crops throughout the year. The mermithid *Ovomermis albicans* (Sieb.) is active only during the rainy season, and only on groundnut and other short statured crops and weeds growing on red soils (Bhatnagar *et al* 1985).

Parasitoids have their preferences for crops irrespective of their host insect. This has been observed not only with sole crops but also with intercrops. Bhatnagar *et al* (1979) observed that parasitoids do not transfer with *H. armigera* from sorghum to pigeonpea in the sorghum/pigeonpea intercrop, but that each crop exhibits its own parasitoid complex.

The average rates of egg and larval parasitism recorded for *H. armigera* over the past eight years on ICRISAT mandate crops at ICRISAT Center are given in table 2. Egg mortalities of up to 33·2% on sorghum, 10·5% on pearl millet, 14·8% on groundnut, 0·3% on pigeonpea, have been recorded. On chickpea, no egg parasitism has ever been recorded.

Most early larval parasitism occurs on pearl millet (50·7%), sorghum (49·5%), and chickpea (31·9%), whereas late larval parasitism occurs chiefly on pigeonpea (16·4%) and groundnut (11·5%). Among larval parasitoids, *C. chlorideae* contributes predominantly to the mortality of 1–3 instar and *C. illota* much to the mortality of 4–6 instar larvae on all crops except groundnut, where, besides these parasitoids, the mermithid *O. albicans* is an equally or more important parasitoid (Bhatnagar *et al* 1985). Although, as a foliage feeder, *H. armigera* causes little or no yield loss in groundnut, the crop may act as an important reservoir for *Heliothis* populations when other hosts are not available or attractive.

The overall rates of egg and larval parasitism of *Heliothis* at ICRISAT Center by month, irrespective of plant species, are given in figure 2. In general, higher rates of parasitism were recorded during the rainy season when *H. armigera* is largely on groundnut, sorghum and pearl millet, whereas lower rates of parasitism were recorded during the postrainy season when *Heliothis* is largely on pigeonpea and chickpea.

Table 1. Parasitoids recovered from *Heliothis* spp. in Andhra Pradesh, Maharashtra and Karnataka 1977–1985.

	Recovered from		
Species	*H. armigera*	*H. peltigera*	*H. assulta*
Insects			
Diptera			
Tachinidae			
Carcelia illota Curran[d]	√	√	√
Exorista xanthaspis Wied[c]	√		
Goniophthalmus halli Mes[d]	√	√	√
Palexorista laxa Curran[c]	√		
Palexorista solennis Walker[c]	√		√
Palexorista sp.[c]	√	√	
Sturmiopsis inferens Tns[c]	√	√	
Hymenoptera			
Bethylidae			
Goniozus sp.[c]	√		
Braconidae			
Apanteles sp.[c]	√		
Bracon sp.[c]	√		
Chelonus sp.[b]	√		
Microchelonus curvimaculatus Cameron[b]	√	√	√
Rogas sp.[c]	√		
Ichneumonidae			
Barichneumon sp.[d]	√		
Campoletis chlorideae Uchida[c]	√	√	
Disophrys[c]	√		
Enicospilus sp. nr. *shinkanus* Uchida[c]	√		
Eriborus argenteopilosus Cameron[c]	√	√	
Eriborus trochanteratus Morley[c]	√		
Ichneumon sp.[c]	√		
Metopius rufus Cam.[c]	√		
Temelucha sp.[c]	√		
Xanthopimpla stemmator Thun.[c]	√		
Trichogrammatidae			
Trichogramma chilonis Ishii[a]	√	√	
Trichogramma sp.	√		
Trichogrammatoidea sp.[a]	√		
Trichogrammatoidea bactrae sp. *fumata* Nagaraja[a]	√		
Mermithid			
Ovomermis albicans (Sieb.)[c]	√	√	√

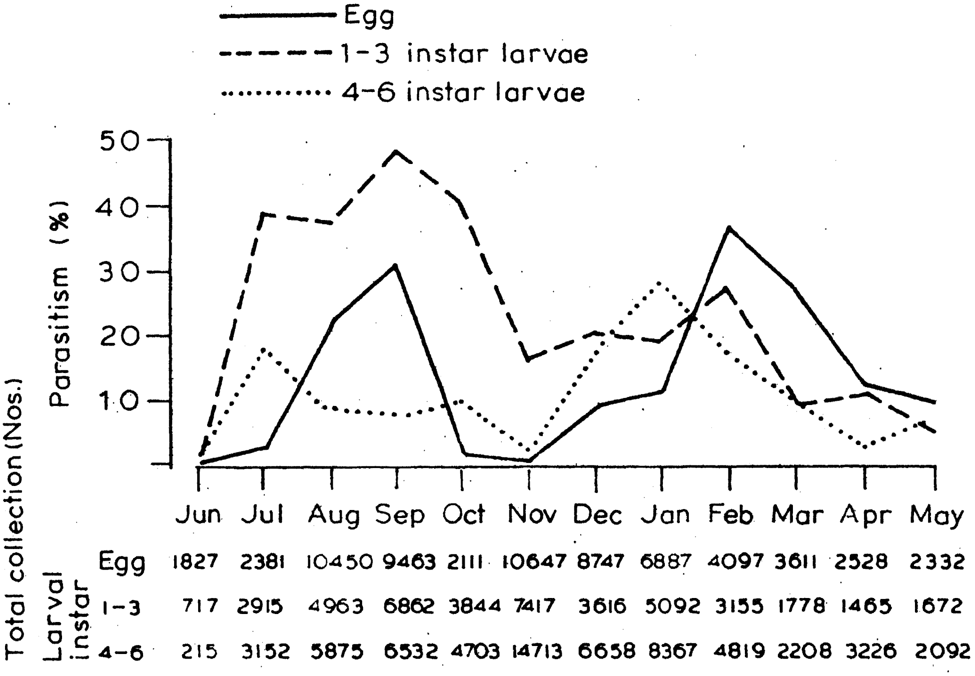
[a,b,c,d] Egg, egg-larval, larval and larval/ex-larval parasitoids respectively.

Table 2. Average parasitism (%) of *H. armigera* eggs and larvae on ICRISAT mandate crops at ICRISAT Center, 1977–1985.

		Larval parasitism (%)			
	Egg parasitism	in 1–3 instars by *Campoletis*		in 4–6 instars by *Carcelia*	
Crops	(%)	Total	Chlorideae alone	Total	Illota alone
Sorghum	33·2 (23511)[a]	49·5 (7877)[a]	45·7	5·8 (8537)[a]	3·9
Pearl millet	10·5 (2986)[a]	50·7 (584)[a]	39·9	5·1 (355)[a]	4·8
Groundnut	14·8 (2778)[a]	14·3[7·4][b] (3492)[a]	6·5	11·5[7·7][b] (3230)[a]	1·5
Pigeonpea	0·3 (21787)[a]	9·9 (10354)[a]	3·4	16·4 (28171)[a]	8·1
Chickpea	0·0 (3700)[a]	31·9 (12969)[a]	31·6	6·1 (13283)[a]	5·7

[a] Total number of collections of eggs or larvae over the years

[b] Nematode parasitism alone (%).



	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Egg	1827	2381	10450	9463	2111	10647	8747	6887	4097	3611	2528	2332
1–3	717	2915	4963	6862	3844	7417	3616	5092	3155	1778	1465	1672
4–6	215	3152	5875	6532	4703	14713	6658	8367	4819	2208	3226	2092

Figure 2. Mean egg and larva parasitism (%) of *H. armigera* at ICRISAT Centre, 1976–85.

4.2 *Predators*

Although 21 insects and 5 spider species have been recorded as predators of *Heliothis* eggs and larvae (table 3) their effect on *H. armigera* population has not yet been quantified. Their activity, however, has been observed to differ with the crop and the soil type. We reared wasps, *Delta* spp., in a field cage and found them active when provided with a pool of water and the sucrose, as a substitute for flowering plants (ICRISAT 1983). Birds have often been recorded feeding on larvae from crops like chickpea in areas where there are trees to serve as roosting sites.

4.3 *Pathogens*

Whereas bacteria, fungi and viruses have sometimes caused mortality to *Heliothis* larvae in the field, little is known about the quantitative impact of pathogens on field

Table 3. Arthropod predators on *H. armigera* (Hb.), recovered in Andhra Pradesh 1977–1985.

Coleoptera	
Coccinellidae	*Menochilus sexmaculatus* F.[ab]
Dermaptera	
Carcinophoridae	*Euborellia annulipes* (Lucas)[bc]
	Euborellia stali Dohrn[bc]
Labiduridae	*Nala lividipes* (Dufour)[b]
Dictyoptera	
Mantidae	*Humbertiella* sp.[bc]
Hemiptera	
Anthocoridae	*Orius* (*Dimorphella*) *maxidentex* Ghauri[ab]
Lygaeidae	*Paromius gracilis* (Rambur)[b]
Nabidae	*Tropiconabis capsiformis* (Germar)[b]
Pentatomidae	*Cantheconidea furcellata* (Wolff)[b]
Reduviidae	*Catamiarus brevipennis* (Serv.)[cd]
	Ectrychotes dispar Reut.[bc]
	Rhinocoris marginatus (Fab.)[bcd]
Hymenoptera	
Eumenidae	*Delta conoideus* G. soyka[cd]
	Delta companiforme esuriens Fabricius[bc]
	Delta pyriformae (Fabricius)[cd]
Sphecidae	*Sphex argentatus* Fabricius[bc]
Vespidae	*Polistes olivaceus* Degeer[cd]
	Ropalidia marginata Lepeltier[cd]
	Vespa orientalis Fabricius[cd]
	Vespa tropica haemotodes Bequaert[cd]
Neuroptera	
Chrysopidae	*Chrysopa* sp.[abc]
Araneida	
Aranidae	*Leucauge tessellata* (Thorb.)[bc]
	Neoseona theis (Walck.)[bc]
Clubionidae	*Clubiona* sp.[bc]
Thomisidae	*Thomisus* sp.[bc]
	Oxyptila reenae (Basu)[bc]

[a, b, c, d] Egg, small larval, medium larval, and large larval predators respectively (indicated based on observations).

populations. However, nuclear polyhedrosis virus (NPV) has severely affected a laboratory culture of *H. armigera* (Bhatnagar *et al* 1982). The potential of NPV on chickpea as a biocontrol agent has been confirmed when artificially applied to chickpea in the field (Bhatnagar *et al* 1983).

5. Scope for biological control of *H. armigera*

In the light of the above observations it could be said that, for biological control of *H. armigera* at and around ICRISAT Center and in similar situations, one must aim at a plan to benefit the crops of pigeonpea and chickpea which are highly vulnerable to *H. armigera*, perhaps for the lack of adequate natural control. Egg parasitism is preferable because the insect is killed before the larva emerges to damage the crop. For example, weekly releases of the egg parasitoid, *T. chilonis*, as practiced by the sugarcane growers in Tamil Nadu, is giving good control of the internode borer, *Chilo sacchariphagous indicus*, of sugarcane (Solayappan 1980). However, it is known that egg parasitoids are not active on pigeonpea and chickpea, so releasing them in these crops is unlikely to be effective. However, releases to increase populations in sorghum, pearl millet, or groundnut to encourage natural control before *H. armigera* transfers to pigeonpea or chickpea may be a possibility. Finding exotic parasitoids which would also prefer *H. armigera* on pigeonpea and chickpea and breeding for crop varieties which are more attractive to natural enemies could also be considered.

Among the larval parasitoids, *C. chlorideae* is a potential candidate for biological control because it parasitises 1–3 instar larvae and is active on almost all crop and weed hosts of *H. armigera* (ICRISAT 1985). Nagarkatti (1982) reported that it is not amenable to mass rearing. A closely related species *C. flavicinta* Ashm. is being considered for introduction into India from the neotropical region (Sankaran 1983). The introduction of such species, however, may not prove wise. When *C. chlorideae* was introduced into the USA it impaired the effectiveness of the native *C. sonorensis* (Cameron) because the two species inbred and produced infertile hybrids (King *et al* 1981).

Attempts to establish a specific larval parasitoid, *Eucelatoria bryani* (Coq.) of *Heliothis*, imported from the USA have failed at ICRISAT Center (ICRISAT 1984) although the National Centre for Biological Control, Bangalore, has reported that it is slowly becoming established around Bangalore (Nagarkatti 1982). The constraint on the establishment of this parasitoid in central India is that it cannot survive temperatures greater than 35°C which are common in the summer (Bhatnagar *et al* 1983). The Indian Council of Agricultural Research (ICAR), New Delhi is now considering introducing the larval parasitoids *Hyposoter didymator* (Thunb.) and *Apanteles kazak* Telenga from Europe where they are reported to check *H. armigera* even under pesticide treated conditions (S P Singh, Perl. Communication). We have to see whether these parasitoids could be established in the country.

The adoption of NPV for the control of *H. armigera* is possible. However, its use at farmers' level has not yet been permitted by the Govt. of India for several reasons including the possibilities of its harmful effects on man and animals. NPV is not effective on all crops; it has been reported to be effective on chickpea (Narayanan 1979; Santharam and Balsubramanian 1982) but not on pigeonpea (Santharam *et al* 1981).

The potential of using predators in biological control of *Heliothis* has been amply demonstrated elsewhere. Ridgway *et al* (1977) obtained good control of *Heliothis* spp. on cotton by periodic releases of eggs and larvae of *Chrysopa carnea* Stephens. The Institute of Agricultural and Forestry Sciences in Shang-Chiu (1976) reported 70–80% reduction in *H. armigera* larval population in cotton fields within 5–7 days of the introduction of colonies of *Polistes* wasps. This type of augmentation of natural enemies could also be attempted here, provided that work on native predators to find their limitations in the manner done for *Delta* wasps at ICRISAT Center is carried out at least for the major predators.

An important consideration for the success of biological control in an IPM Program is the use of insecticides that are relatively less toxic to parasitoids and predators than to the pests (Croft and Brown 1975). This, however, calls for the testing of available insecticides against, at least, the major parasitoids and predators as is being done in the developed countries.

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Testicular damage in rats fed on irradiated diets

A K S KUSHWAHA and S S HASAN
Department of Animal Science, Faculty of Life Sciences, Rohilkhand University, Bareilly 243 001, India

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Abstract. Feeding effect of irradiated diets was studied on the pups born to mother fed either on irradiated normal diet or irradiated low protein diet. The study indicated that pups born to mother fed on the irradiated low protein diet had fewer spermatogonial cells in the testes than those given irradiated normal diet and unirradiated low protein diet. Similarly, pups maintained on the irradiated low protein diet showed marked decrease in alkaline phosphatase and cholesterol contents in the testes rather than in the pups fed irradiated normal as well as unirradiated low protein diets. The irradiated low protein diet fed pups showed increased depletion and vacuolization of adrenocortical and medullary cells.

Keywords. Adrenal; testes; irradiated diet.

1. Introduction

Since about over a quarter of our harvested food is lost due to various kinds of wastage and spoilage, food preservation is no less important than food production. More recently many of the developing nations of the world have realized the potential offered by irradiation for reduction of the tremendous losses which occur after harvest (Kampelmacher 1980). But the consumption of irradiated food seems to be associated with greater risk in malnourished population. Malnourished rats when switched over to irradiated wheat were found to have significantly fewer germ cells than those given irradiated wheat diet, besides, malnourished rats given irradiated wheat showed more intrauterine death than malnourished rats given unirradiated wheat (Vijayalaxmi and Visweswara Rao 1976). On the contrary, Iwado *et al* (1972) have demonstrated feeding of irradiated diets to show a reasonable reproduction rate through 3 successive generations and the growth rate and diet consumption were greater in young animals fed on irradiated diets than in those fed auto-claved diets. Thus the contradictory findings do exist regarding impact of irradiated diets on the germinal cells and growth of the animals. Similarly, as a sequal to work carried out in the past it has been established that ionizing radiation decreases production of corticoids from the adrenal glands (Berliner *et al* 1962; Nabors 1962; Nabors *et al* 1974; Stevens *et al* 1963) and the response of adrenal activity is mostly dose dependent to radiation (Dougherty and White 1964; French *et al* 1955).

However, there is no information on the developing adrenals of rats given either irradiated normal diet or irradiated low protein diet during gestation and lactation. In the present investigation development of testes in terms of its weight, cholesterol and alkaline phosphatase contents was studied on the pups born to mothers fed on the irradiated normal diet (consisted equal parts of wheat and gram) and irradiated low protein diet (consisted one part of normal diet and 3 parts of wheat). A histological study was also undertaken to assess the amount of damage caused in the adrenal and testes of pups fed either on irradiated normal or irradiated low protein diets.

2. Materials and methods

This experimental study was conducted on inbred rats of Holtzman strain. Virgin female rats weighing 180–200 g were housed with male rats in breeding cages with free access of food and water. After 24 h female rats were examined for the presence of a vaginal plug, or a vaginal smear and this was done to confirm the presence of sperms as an indication of mating. On the 4th day rats were randomly divided into 4 groups and were housed under identical animal husbandry conditions.

Group I: 10 rats received unirradiated normal diet (consisted equal parts of wheat and gram).
Group II: 10 rats were fed irradiated normal diet.
Group III: 10 rats were given unirradiated low protein diet (consisted one part of normal diet and 3 parts of wheat).
Group IV: 10 rats were fed irradiated low protein diet.

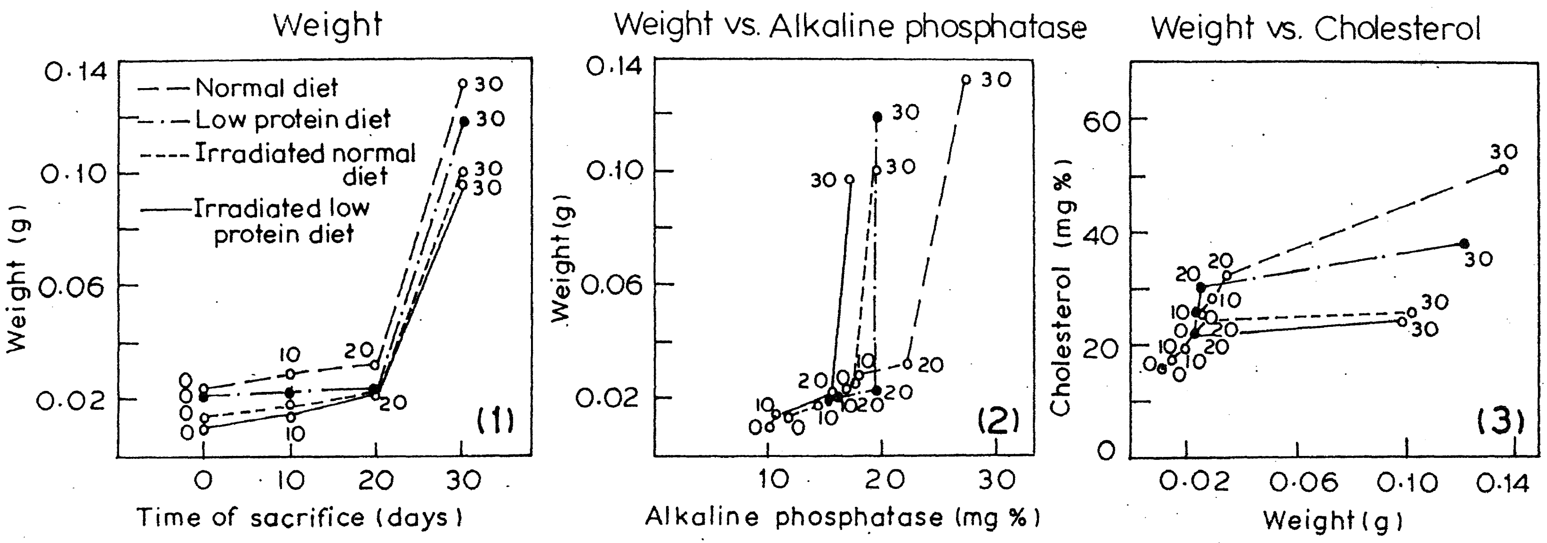
Irradiated diets were used within one month after irradiation. Wheat and gram used were of identical batch of control material and were milled to a course flour and used in the preparation of diets. Feeding of both irradiated and unirradiated diets was started from the 3rd day of post-irradiation. Wheat and gram were separately soaked in water for 48 h before being irradiated periodically in 1·25 kg lots in aluminium containers. The containers were exposed to 10,000 R, x-rays at dose rate 80 KV, 100 MAS, distance 40 cm, time 2 s. Simultaneously the unirradiated wheat and gram for control diet were also soaked in water for 48 h to serve as control for irradiated diets.

Each rat was given 5 g of mashed diet every evening. Any food left from the previous day was removed. The animals received their allotted diets throughout gestation, lactation and entire tenure of the experiment. Water was given *ad libitum*. Number of pups delivered in each litter varied from 3–8. There was lag phase of one month between the irradiation and the time when the animals were given this food.

Six young pups from each group were killed at the following stages of development: at birth, 10, 20 and 30 days after birth. The rats were killed by decapitation and their testes were quickly removed and 20% homogenates of these testes were prepared in sucrose solution. Homogenates were used to estimate alkaline phosphatase (Saekett 1960) and cholesterol (King and Wootton 1959). A few lobes of testes and adrenal were fixed in Bouin's fluid for histological examination. Five micra-thick paraffin sections were cut and stained in haematoxyline and eosine.

3. Results and discussion

It is observed that feeding of irradiated diet decreases the activity of androgen sensitive enzyme alkaline phosphatase (figure 2) and cholesterol (figure 3) in the testes. Changes appear to be more pronounced in the testes of pups fed irradiated low protein diet rather than in those pups given irradiated normal diet. These changes are revealed by more severe depression in the level of alkaline phosphatase

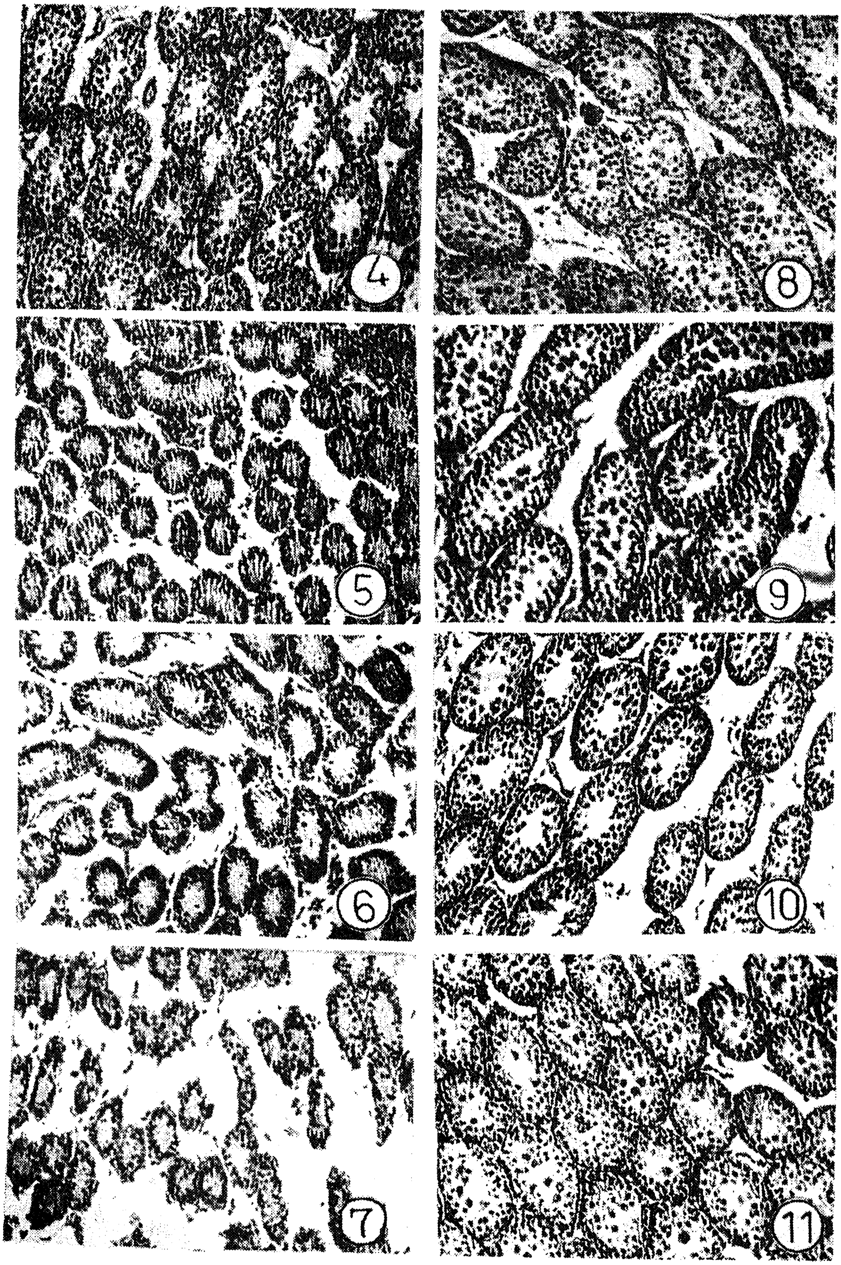


Figures 1–3. **1.** Showing the levels of testes weight during post-natal development. **2.** Showing the activity of alkaline phosphatase in the testes during post-natal development. **3.** Showing the levels of cholesterol in the testes during post-natal development.

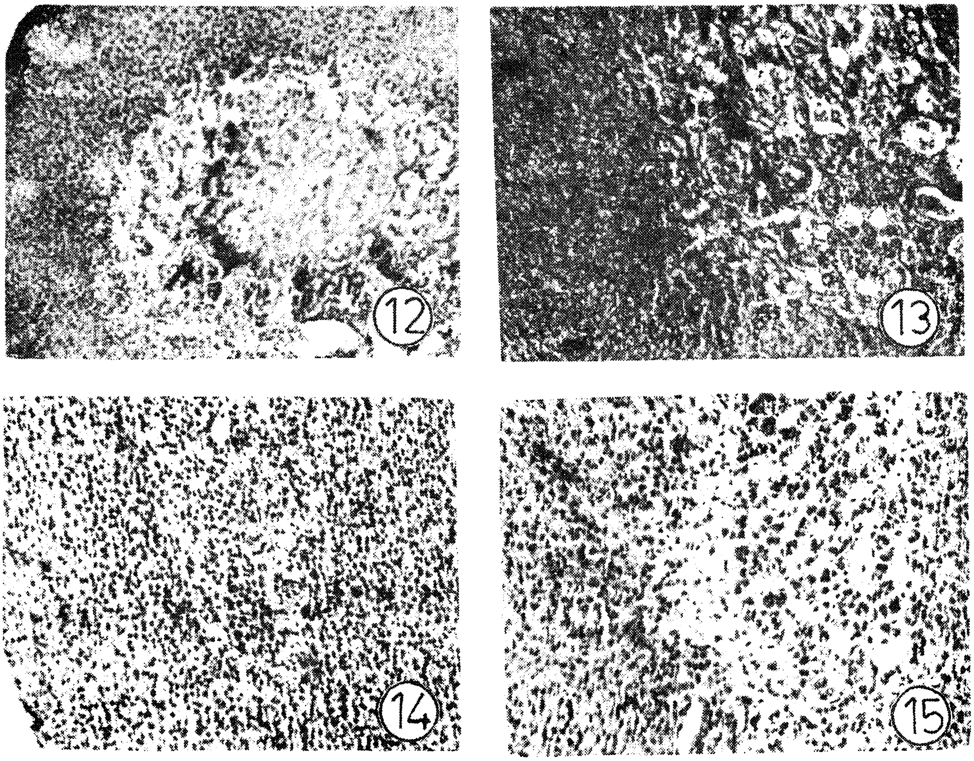
and cholesterol (figures 2 and 3) contents of testes of pups fed irradiated low protein diet compared with irradiated normal diet fed pups. Pups fed on the unirradiated low protein diet had higher alkaline phosphatase and cholesterol (figures 2 and 3) contents in the testes than in the pups fed irradiated normal diet and irradiated low protein diet. Similarly, the growth of the testes recorded in terms of testicular weight (figure 1) showed a sharp decline in pups given irradiated low protein diet than in the irradiated normal diet. On the contrary, pups born to mothers fed on the unirradiated low protein diet showed an increase in growth of testes compared with pups fed irradiated normal diet and irradiated low protein diet (figure 1). However, the growth rate of testes was lower in the pups fed on the unirradiated low protein diet than that was observed in the normal diet fed control pups (figure 1). The data recorded on the growth rate of testes corroborate the biochemical findings on alkaline phosphatase and cholesterol contents of the testes. This suggests that the feeding of irradiated diet interferes with the development of testes possibly due to formation of toxic substances 'radiotoxins' in the diet by irradiation. The more severe decrease in enzyme activity of alkaline phosphatase and cholesterol contents of the testes of the pups born to mother fed on the irradiated low protein diet indicates the reduced availability of protein and other co-factors besides formation of toxic substances.

The biochemical findings are further substantiated by the histological studies on the testes (figures 4–11). Irradiated diet fed pups showed a marked atrophy in the size of seminiferous tubules and this was accompanied by subsequential reduction in the number of spermatogonial cells (figures 6, 7 and 10). Population of spermatogonial cells was remarkably lower in the irradiated low protein diet fed pups (figures 7 and 11) than those given irradiated normal diet (figures 6 and 10). Vijayalaxmi and Visweswara Rao (1976) have also reported that the malnourished rats when switched over to irradiated wheat diet which consists of 9% protein, show significantly fewer germ cells than the well fed animals given the same irradiated wheat diet. This suggests that the feeding of irradiated diet is associated with some reduction in the number of germ cells. The results of this study indicates that feeding of irradiated low protein diet results in reduced number of germ cells in the testes (figures 7 and 11) than those given irradiated normal (figures 6 and 10) and unirradiated low protein diets (figures 5 and 9). Thus the present investigation show the testicular depletion after feeding irradiated diet. The increased intrauterine death and reduction in number of spermatogonial cells reported in this study might have partly been contributed by decrease in the synthesis of androgen sensitive enzyme alkaline phosphatase and cholesterol contents in the testes of irradiated low protein diet fed pups.

The results of this study further reveal that the feeding of irradiated diets to rats during gestation and lactation causes increased depletion of granular contents in the cytoplasm of cortical and chromaffin cells of adrenal (figures 14 and 15), compared with the pups fed on the unirradiated normal diet and unirradiated low protein diet (figures 12 and 13). The increased depletion and appearance of vacuoles in the cells demonstrate an increase in cellular activity of adreno-cortical and medullary cells. This probably indicates increased metabolism of hormones produced from cortical and chromaffin tissue due to production of toxic substances by irradiation of diet. Wolf (1973) in his experiment reported the toxic substance radiotoxins to be formed in diet by irradiation. The extent of increase in the cellular activity was greater in the adrenals of pups given irradiated diet rather than those fed unirradiated normal diet.



Figures 4–11. Photomicrographs of TS of testes of normal control and experimental rats, Haematoxylin and Eosin (× 100). **4 and 8.** Unirradiated normal diet fed pups, 20th and 30th day of life showing blood vessels, connective tissue and interstitial cells of leydig between the seminiferous tubules. **5.** Unirradiated low protein diet fed pups, 20th day of life showing decrease in size of seminiferous tubules. **6 and 10.** Irradiated normal diet fed pups, 20th and 30th day of life showing spermatogonial cells bordering the epithelium of the tubules. **7.** Irradiated low protein diet fed pups, 20th day of life showing marked reduction in the number of seminiferons tubules. **9.** Unirradiated low protein diet fed pups, 30th day of life showing decrease in the number of tubules. **11.** Irradiated low protein diet fed pups, 30th day of life showing lesser number of spermatogonial cells.



Figures 12–15. Photomicrographs of TS of adrenal gland of rats, H and E (× 100). **12.** Unirradiated normal diet fed pups, 30th day of life showing granulated cells in both cortex and medulla. **13.** Unirradiated low protein diet fed pups, 30th day of life showing irregular strands and masses of cells separated by sinusoidal vessels in the cortex. **14.** Irradiated normal diet fed pups, 30th day of life showing appearance of vacuoles in the adreno-cortical cells. **15.** Irradiated low protein diet fed pups, 30th day of life showing vacuolated adreno-cortical and medullary cells.

Vijayalaxmi and Sadasivan (1975) have reported that rats maintained on the low levels of dietary protein perse can produce structural abnormalities. Thus it is evident from the present study that irradiation of diet brings about some qualitative change in the diet which is associated with the increase in cellular activity of the adrenal cortex and medulla of rats.

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Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura)*

C A NAGESWARA RAO**, K SHYAMA SUNDARI and
K HANUMANTHA RAO
Department of Zoology, Andhra University, Waltair 530 003, India
**Zoological Survey of India, Hillpatna, Berhampur 760 005, India

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Abstract. Wide fluctuations in the concentration of the haemolymph protein and its copper were observed during the moult cycle of the crab *Ocypoda macrocera*. Blood protein and copper concentrations were more in the intermoult (C_4) and premoult crabs (D_1–D_2) but decreased in the postmoult crabs (A_1–B_2). A direct relationship between the blood protein and copper concentration is observed during the moult cycle. More copper in the hepatopancreas is seen during the premoult stage (D_1–D_2) and its level increased further in the postmoult stages (A_1–B_2). Increase of hepatic copper with a decrease in haemolymph copper content during the post moult stages shows the transfer of copper liberated from the haemolymph to the hepatopancreas due to the breakdown of haemocyanin, the major blood protein, during starvation period of moult cycle.

Keywords. *Ocypoda macrocera*; moult cycle; haemolymph; hepatopancreas; blood protein; copper.

1. Introduction

The moult and reproductive cycles of the crustaceans are accompanied by physiological changes including variations in haemolymph proteins. Extensive studies on the physiological changes occurring in the crustacean blood associated with reproduction, moulting, starvation etc have been reported by Barlow and Ridgway (1969), Glynn (1968), Busselen (1970), Djangmah (1970), Djangmah and Grove (1970), Dall (1974) and Hepper (1977). Haemocyanin, the copper containing protein of the crustacea is reported to occur freely in the haemolymph and it has also been reported that it is the major blood protein fraction (Goodwin 1960). In addition to haemolymph, hepatopancreas of crustaceans has been found to be the only tissue which contains considerable amounts of copper (Kerkut *et al* 1961).

In the present investigation, the total blood protein and copper levels of haemolymph of the commonly occurring ghost crab *Ocypoda macrocera* during its moult cycle has been studied to understand the changes in protein and copper concentration of blood during moult cycle. The copper concentration of hepatopancreas during the moult cycle has also been studied to assess the haemolymph and hepatopancreas copper relationship.

2. Materials and methods

Specimens of *O. macrocera* were collected from the Lawson's Bay and palm beach areas of Visakhapatnam coast. Crabs were maintained separately in glass jars containing sea water (for submersion of the branchial region) and were fed with fish muscle and the sea water was changed daily. Crabs were found to be quite active for longer time (45–60 days) under laboratory conditions and several crabs moulted. Many of the crabs were seen attaining the C_4 stage after their moult in the laboratory. Male crabs measuring 1·8–2·2 cm carapace width, which have shown high moulting activity were taken in the present experiment. The moult stages were distinguished after Drach (1939) and Nagabushanam and Ranga Rao (1960). Blood samples from the intermoult and other moult stages were collected through a hypodermic syringe from the pericardial space and also from the joints of chelate legs. The total blood protein and blood copper estimations were made using whole blood. Hepatopancreas samples collected from different moult stages were dried, powdered and the same were taken for hepatic copper estimation. Total blood protein was determined by the method of Lowry *et al* (1951). Blood and hepatopancreas copper quantities were estimated following the oxalyldihydrazide method of Rice (1960) and Ravindranath (1981). Samples of haemolymph and hepatopancreas for each moult stage were taken from several crabs and the values presented in the results for each reading is the mean of 6–8 observations.

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3. Results and observations

The mean total blood protein at different moult stages of *O. macrocera* is shown in table 1. Normal intermoult crabs (C_4) collected from the same locality showed considerable variations in their blood protein concentration and it ranged between 64·05–93·63 mg/ml, and a mean value of 81·58 mg/ml was noted. In the premoult crabs (D_1–D_2) blood protein ranged between 94–132 mg/ml and a mean value of 112·50 mg/ml was recorded. A falling blood protein quantity is noticed in the A_1 stage with 69·73 mg/ml (51·75–81·32). Further decline in the total blood protein quantity is noticed in the B_2 stage with a value of 31·12 mg/ml (21·74–46·41). The revival in the blood protein concentration is noted in the C_2 stage with 43·96 mg/ml (30·93–62·03). Increase in the blood protein level continued and in C_3 stage 66·44 mg/ml of protein (50·76–78·04) was noted.

The blood copper concentration also showed wide variations among different crabs within the same population. The results on the blood copper concentration among different moult stages are shown in table 1. Haemolymph copper concen-

Table 1. Total haemolymph protein, haemolymph copper and hepatopancreas copper quantities in different moult stages of *O. macrocera*.

Moult stage	Haemolymph protein (mg/ml) ± SD	Haemolymph copper (μg/ml) ± SD	Hepatopancreas copper (μg/g dry weight) ± SD
C_4	81·58 ± 12·07	48·78 ± 11·31	299·44 ± 70·34
D_1–D_2	112·50 ± 16·36	79·55 ± 16·48	385·00 ± 68·44
A_1	69·73 ± 11·08	30·01 ± 9·92	475·33 ± 61·33
B_2	31·12 ± 8·26	18·65 ± 6·27	501·47 ± 40·37
C_2	43·96 ± 15·77	28·03 ± 10·24	411·18 ± 59·16
C_3	66·44 ± 13·08	37·60 ± 8·19	406·52 ± 51·91

SD = Standard deviation.

tration values showed a similar pattern in its variations during the moult cycle as those seen in the case of blood protein.

Intermoult stage (C_4) crabs showed haemolymph copper of 48·78 μg/ml (27·75–63·32) and an increase of haemolymph copper is found in the premoult (D_1–D_2) stages with blood copper concentrations of 79·55 μg/ml (54·55–101·76). Decrease in haemolymph copper concentration is noted in all post moult stages. It is observed that with an increase in blood protein there is a corresponding increase in blood copper concentration, thus showing a direct relationship between haemolymph protein and its copper during moult cycle (figure 1).

Quantity of copper in the hepatopancreas also showed wide variation among crabs of the same population and of similar moult stages. The quantities of hepatic copper found in different moult stages of *O. macrocera* is shown in table 1. The changes in the hepatic copper shows that hepatic copper values increased from premoult (D_1–D_2) stage to early post moult (B_2) and a sharp decrease is found in C_2 and further decrease in C_3 stage is noticed thus reaching to normal level by C_4 stage.

4. Discussion

Busselen (1970) reported a 4-fold decrease in haemocyanin concentration after moult in *Carcinus maenas* and he observed that copper concentration of the blood serves as a measure of the haemocyanin concentration. Negligible amounts of blood copper was reported in C_1 stages of *Maia squinado* (Zuckerkandl 1960). Decrease in blood copper was noticed in late post moult stages of *C. maenas* and *Crangon vulgaris* by Kerkut *et al* (1961) and Djangmah (1970) respectively.

The fall of blood protein in post moult stages is attributed to the absorption of large quantities of water and protein utilization during the moult cycle. Similar to the present observation, a direct relationship of blood protein and copper quantities during moult cycle has also been reported in *C. vulgaris* by Djangmah (1970). The fluctuations of hepatic copper during the moult cycle of *O. macrocera* has shown an

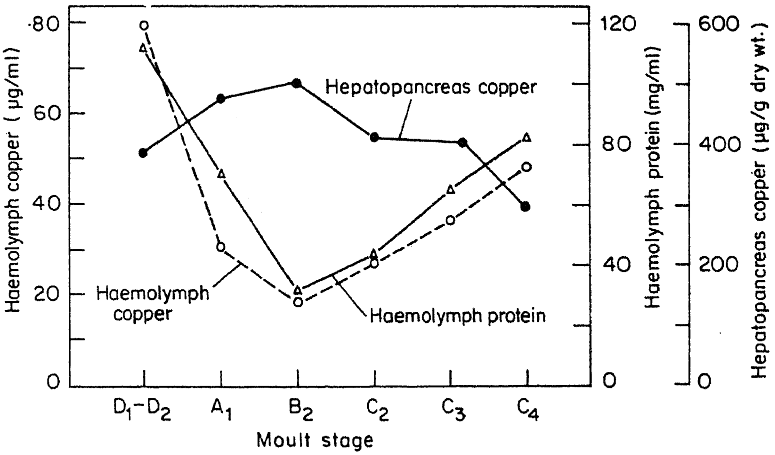

Figure 1. Haemolymph protein, copper and hepatopancreas copper concentrations during moult cycle.

inverse relationship with that of blood copper i.e. with a decrease of blood copper an increase in hepatic copper was observed (figure 1). Zuckerkandl (1960) reported an increase in hepatic copper in the early post moult stages and its decrease in the late post moult stages of *Maia squinado* and he indicated re-entry of hepatic copper into blood in the late post moult stages. Djangmah (1970) reported an increase in hepatic copper and decrease in blood copper during starvation in *C. vulgaris* and he observed that some amount of the liberated copper from haemolymph is absorbed into the hepatopancreas. However he indicated that all the absorbed copper of hepatopancreas is not retransferred for haemocyanin reconstitution.

Djangmah and Grove (1970) did not report any correlation between the blood copper and of hepatopancreas copper during the moult cycle in *C. vulgaris*. Contrastingly Kerkut *et al* (1961) reported an increase in hepatic copper with simultaneous increase of blood copper.

In the present investigation decrease in blood copper in the early post moult stages with a simultaneous increase in hepatic copper indicates transfer of copper from blood to hepatopancreas. Further the increase in haemolymph copper during the late post moult stages evidently shows some copper re-entering blood in the post moult stage as it is found that the blood copper level increased in the early C_2 stage, even before the crab is supplied with food material.

There is no reported evidence on the re-entry of hepatic copper into the haemolymph for the reconstitution of haemocyanin during late post moult stages; while there are reports of copper loss through faeces in the form of copper bodies in *Procambarus clarkii* by Ogura (1959). Similarly Bryan (1964, 1968) also indicated the loss of zinc through faeces and urine in *Homarus vulgaris* and *Austropotombius pallipes*. The finding in the present study shows that certain amount of the copper absorbed from the haemolymph into the hepatopancreas during the early post moult stages is reabsorbed into the haemolymph of the late post moult stage crabs.

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Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch)

K R S SAMBASIVA RAO and K V RAMANA RAO*
Department of Zoology, Nagarjuna University, Nagarjunanagar 522 510, India
*Department of Zoology, SVUPG Centre, Kavali 524 202, India

MS received 7 November 1985

Abstract. *Channa punctatus*, exposed to sublethal concentration of phenthoate showed a significant decrease of glycogen content under both static and continuous exposure systems. Further, succinate, malate and lactate dehydrogenases in the muscle, gill and liver tissues showed decreased activity. The inhibition of these enzyme systems was relatively more in continuous flow than in static media. Results indicate that phenthoate reduces the tissue oxidative status of the fish both in static and continuous flow systems. This impact is more conspicuous with the continuous flow system which may be attributed to the continuous availability of the pesticide whereas in the static media pesticide detoxification is possible with progress of time.

Keywords. Phenthoate; continuous flow; static media; respiratory enzymes; *Channa punctatus*

1. Introduction

It has been well established that pesticides after getting translocated reach both the stagnant waters like ponds as well as the rivers where they exhibit differential toxicity (Matsumura *et al* 1972; Rao *et al* 1981). Since rate of flow exhibits considerable influence on the toxicity of a given compound (Edwards 1973), a study of toxic influence emanated by both static and continuous flow systems present best examples to have relative understanding of the media effect. In view of this, an attempt was made in the present study to correlate the toxic impact of phenthoate (a widely used organophosphate pesticide) in static and continuous flow media on the freshwater fish, *Channa punctatus* Bloch. Because of the regressive response of dehydrogenases to insecticides observed earlier (Sahib 1979; Rao 1980), these enzymes were also assayed in the present study.

2. Materials and methods

The details of collection, maintenance and acclimatization of the fish, *C. punctatus* were described earlier (Rao *et al* 1981). The commercial grade phenthoate was used in the present study. A stock solution of 1000 ppm was prepared by dissolving phenthoate in small quantity of acetone and diluted with water to obtain the required concentrations. Acetone used in the present study (0·5 ml) is non-toxic to the fish (Pickering *et al* 1962). The LC_{50} of phenthoate to the fish was determined by probit analysis (Finney 1971) and the 48 h LC_{50} was found to be 0·43 ppm. One third of the LC_{50} value (0·15 ppm) was taken to represent sublethal concentration for further experiments (Konar 1969). The fish were divided into batches of 6 each, one batch was exposed to 0·15 ppm phenthoate in static media for 48 h and another batch was exposed to the same concentration in continuous flow system. Suitable controls were maintained without the addition of pesticide.

2.1 *Design for continuous flow system*

In the continuous flow system, freshly prepared sublethal concentration of phenthoate was allowed to pass in and out of a 50 L aquaria at a flow rate of 250 ml/min. To maintain the concentration of the chemical constant, mechanical stirrers were used to avoid any possible fluctuations in the medium. Also the troughs containing the fish were aerated to prevent hypoxic or anoxic conditions in the medium. Controls also received similar treatment.

After exposure, the control and phenthoate exposed (PE) fish was sacrificed and the tissues like muscle, gill and liver were isolated and kept in cold for the estimation of glycogen and dehydrogenases. The glycogen content was estimated by the method of Carrol *et al* (1956). Succinate dehydrogenase (succinate: acceptor oxidoreductase EC 1·3·99·1) and malate dehydrogenase (L-malate: NAD oxidoreductase EC 1·1·1·37) activities were estimated as per the method outlined by Nachlas *et al* (1960) and lactate dehydrogenase (L-lactate: NAD oxidoreductase EC 1·1·1·27) by the method suggested by Srikanthan and Murthy (1955). The values were subjected to statistical analysis using student's *t*-test (Bahn 1972).

3. Results and discussion

The glycogen content and the activity levels of succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) of muscle, gill and liver tissues of PE fish in both continuous flow and static media showed a significant decrease and the inhibition of these enzyme systems is relatively more in continuous flow than in static media (tables 1 and 2).

The decrease in the glycogen content in the 3 selected tissues of PE fish evidently suggests the greater utilization of this substance to meet high energy demands to mitigate pesticide toxic stress. A similar decrease in glycogen content in the tissues of *Tilapia mossambica* exposed to methyl parathion (Rao and Rao 1979) and malathion (Sahib *et al* 1983) was reported. The decrease in glycogen content of PE fish tissues may be due to stimulation of hormones that accelerate glycogen breakdown or inhibition of those associated with glycogen synthesis (Sahib *et al* 1983). The decrease in glycogen is relatively more associated with continuous flow system, which envisages that continuous flow system is more effective in terms of toxicity. Since anoxia or hypoxia are known to increase carbohydrate consumption (Dezwaan and Zandee 1972), the depletion in glycogen content and consequent decrease in respiratory enzymes (tables 1 and 2) during PE exposure suggest the prevalence of hypoxic condition.

The decrease in SDH activity corroborates with the depletion in glycogen content of PE exposed fish tissues. The decrease in SDH activity suggests a reduction in the rate of oxidative metabolism at mitochondrial level possibly resulted by a change in ultrastructure of mitochondria (Vasilos 1976). Since SDH has a greater regulatory influence on the other oxidative enzymes, the reduction in the SDH activity might depress other oxidative enzymes (Lehninger 1978). Thus, MDH activity showed a concomitant decrease both in static and continuous flow systems.

Table 1. Levels of glycogen (mg/g wet wt of tissue) and succinate dehydrogenase (μ mol of formazan formed/mg protein/h) activity in control and PE fish tissues under static and continuous flow systems.

	Static media		Continuous flow	
Tissue	Control	PE	Control	PE
Glycogen				
Muscle	4·72 ± 0·67	2·79 ± 0·21 (41)*	4·89 ± 0·83	2·16 ± 0·27 (56)*
Gill	1·97 ± 0·13	1·01 ± 0·10 (49)*	1·65 ± 0·11	0·75 ± 0·06 (55)*
Liver	30·15 ± 2·23	14·31 ± 1·63 (53)*	28·63 ± 1·11	11·62 ± 1·56 (59)*
Succinate dehydrogenase				
Muscle	0·526 ± 0·039	0·340 ± 0·030 (35)*	0·513 ± 0·050	0·319 ± 0·050 (38)*
Gill	0·430 ± 0·042	0·310 ± 0·041 (28)*	0·414 ± 0·037	0·300 ± 0·023 (28)*
Liver	0·531 ± 0·053	0·320 ± 0·021 (40)*	0·527 ± 0·042	0·312 ± 0·036 (41)*

Values are mean ± SD of 6 individual observations.
Values in parentheses are percent inhibition over control.
*Significantly different from control at $P < 0{\cdot}05$.

Table 2. Levels of malate and lactate dehydrogenase (μ mol of formazan formed/mg protein/h) in control and PE fish tissues under static and continuous flow systems.

	Static media		Continuous flow	
Tissue	Control	PE	Control	PE
Malate dehydrogenase				
Muscle	0·104 ± 0·016	0·073 ± 0·009 (21)*	0·105 ± 0·019	0·070 ± 0·008 (33)*
Gill	0·101 ± 0·011	0·067 ± 0·007 (33)*	0·096 ± 0·007	0·057 ± 0·008 (40)*
Liver	0·283 ± 0·013	0·176 ± 0·034 (38)*	0·238 ± 0·020	0·113 ± 0·016 (52)*
Lactate dehydrogenase				
Muscle	0·526 ± 0·039	0·340 ± 0·029 (35)*	0·513 ± 0·049	0·319 ± 0·046 (38)*
Gill	0·430 ± 0·042	0·310 ± 0·041 (28)*	0·414 ± 0·037	0·300 ± 0·023 (28)*
Liver	0·531 ± 0·053	0·320 ± 0·021 (40)*	0·527 ± 0·072	0·312 ± 0·066 (59)*

Values are mean ± SD of 6 individual observations.
Values in parentheses are percent inhibition over control.
*Significantly different from control at $P < 0{\cdot}05$.

The decrease in NAD dependent LDH activity indicates the accumulation of lactic acid. Since lactic acid accumulation envisages a hypoxic condition (Mayes 1977), the increase in accumulation of lactic acid may contribute partially to the inactive condition of the fish during toxic stress. The reduced activity levels of dehydrogenase systems in all the 3 tissues explicitly show impairment of oxidative metabolism. Hence, it can be inferred that phenthoate toxicity results in low energy state in tissues of the fish, which necessitates these tissues to take advantage of their specialised physiological functions to derive energy under toxic stress.

From these results, it is evident that phenthoate reduces the tissue oxidative status of the fish both in static and continuous flow systems. However, phenthoate impact through continuous flow system showed greater effect than in static medium. This may be due to continuous availability of pesticide in the medium, resulting in the increased rate of pesticide entry into the fish. Whereas in static media, the possibility of pesticide detoxification with lapse of time is more.

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Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation

G K KULKARNI*, A BHASKAR RAO and C S K ANAND
Department of Zoology, Marathwada University, Aurangabad 431 004, India

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Abstract. Hormonal involvement in the control of blood glucose level of *Poecilobdella viridis* was tested by performing debraining, supra- and sub-oesophageal ganglionectomy, followed by injections of the extract of neuroendocrine tissues in operated leeches. Anatomically, the brain ring, through which the oesophagus passes, of this leech is composed of two separate ganglia, the supra-oesophageal and sub-oesophageal, connected to each other by circum-oesophageal connectives. In normal leeches blood glucose level was found to be 10·2 μg%. Removal of complete brain and only the supra-oesophageal portion of the brain produced after 24 h a significant ($P < 0{\cdot}01$) decrease in the blood glucose concentration. Sub-oesophageal ganglionectomy produced no effect. Injections of brain and supra-oesophageal ganglion extracts into 24 h debrained and supra-oesophageal and sub-oesophageal ganglionectomized leeches increased the blood glucose level significantly, but the injections of sub-oesophageal ganglion homogenates had no effect. These data indicate that supra-oesophageal ganglionic portion of the brain of *Poecilobdella viridis* may be the production site of hyperglycemic hormone.

Keywords. Blood glucose; neuroendocrine regulation; supra-oesophageal ganglion; hyperglycemic hormone.

1. Introduction

Sugars in some of annelids have been analysed and generally measured as reducing sugars like glucose and certain disaccharides, such as maltose (Oglesby 1978). Clark (1964) found 72–264 mg/100 ml of total carbohydrates in the coelomic fluid of the polychaete, *Nephtys hombergi* and reported that glucose was the major and maltose was the minor component. Hanumante (1975) also reported that 14·5 mg% of blood glucose present in an earthworm, *Perionyx excavatus*. However, the reports on blood glucose levels in hirudinean annelids are very sparse, except a few on the free sugar levels of body fluids in *Glossiphonia complanata* and *Helobdella stagnalis* (Florkin and Scheer 1969).

In *Poecilobdella viridis*, various physiological phenomena, like respiration, osmoregulation, nitrogen excretion and digestion (Bhaskarrao 1983) and gametogenesis (Nagabhushanam *et al* 1976; Kulkarni and Nagabhushanam 1980) were already found to be under the influence of brain hormone. The present study is undertaken to determine the blood glucose level and the role of brain hormone, if any, in the regulation of blood glucose level of the freshwater leech, *P. viridis*.

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*To whom all correspondence should be addressed.

2. Materials and methods

The leeches, *P. viridis* used for experiments were adult and healthy, having approximately equal size (15–16 cm) and weight (8–10 g), collected from the freshwater ponds around Aurangabad city. In the laboratory leeches were maintained in glass troughs containing wet mud and kept under normal day/night illumination at 25 ± 0·5°C. A 3 days pre-experimental period was allowed to get leeches acclimatized to the prevailing laboratory conditions. To determine the blood glucose levels and to ascertain the role of brain as a whole and of its components like supra- and sub-oesophageal ganglia hormones individually in regulating the blood glucose level, leeches were divided into two groups with following protocol.

Group-I: It was further divided into 7 sub-groups, each of 5 leeches.
(i) Normal control (brain intact).
(ii) 24 h sham operated controls (brain was exposed, slightly agitated but left intact).
(iii) 24 h debrained.
(iv) 24 h debrained leeches received 2 injections of distilled water after every 6 h (50 μl/leech).
(v) 24 h debrained leeches received 2 injections of whole brain extract after every 6 h (50 μl = 2 brains/leech).
(vi) 24 h debrained leeches received 2 injections of supra-oesophageal ganglion (SPOG) homogenate after every 6 h (50 μl = 2 SPOG/leech).
(vii) 24 h debrained leeches received 2 injections of sub-oesophageal ganglion (SOG) extract after every 6 h (50 μl = 2 SOG/leech).
In addition to the first two sub-groups of Group-I, the Group-II was further divided into 4 more sub-groups and each sub-group contained 5 leeches.

Group-II
(i) 24 h supra- and sub-oesophageal ganglionectomized leeches.
(ii) 24 h supra- and sub-oesophageal ganglionectomized leeches received 2 injections of distilled water after every 6 h (50 μl/leech).
(iii) 24 h supra- and sub-oesophageal ganglionectomized leeches injected with 2 injections of supra-oesophageal ganglion homogenate (50 μl = 2 SPOG/leech) after every 6 h.
(iv) 24 h supra- and sub-oesophageal ganglionectomized leeches administered with 2 injections of sub-oesophageal ganglion extract (50 μl = 2 SOG/leech) after every 6 h.

In leeches it is already reported that blood circulation takes place through 4 main longitudinal coelomic sinuses: a dorsal sinus above the gut, a ventral sinus covering the ventral nerve cord and anterior and posterior ganglionic masses, and two lateral sinuses (Bhatia 1977). In order to determine the blood glucose levels of *P. viridis* from both control and experimental groups leeches were dissected after the stipulated time period by taking an incision latero-dorsally all along the body length and exposed the sinuses. 0·25 ml of blood was taken out directly from these sinuses with the help of a hypodermic syringe, having 27 gauze needle and 0·1 ml of blood was then transferred to separate test tubes. The methods of deproteinization and estimation of blood glucose using photoelectric colorimeter was the same as described by Oser (1976). Results of 3 replications were averaged and blood glucose levels were represented as μg%.

3. Results

Data from figure 1 clearly show that an average blood glucose level in normal leeches was found to be 10·2 µg%. 24 h debrained leeches showed a significant ($P<0·01$) decrease in their blood glucose concentration as compared to the sham operated controls. 24 h debrained leeches when administered with the whole brain homogenate produced a quite remarkable increase in blood glucose level as compared to the values of 24 h debrained leeches injected with distilled water. Supra-oesophageal ganglion homogenate alone when administered into the brainless leeches, a significant ($P<0·01$) increase in the blood glucose level was observed as compared to the brainless leeches injected with distilled water. The extracts of sub-oesophageal ganglion when injected into the brainless leeches could not produce any effect.

24 h supra-oesophageal ganglionectomized leeches showed a significant ($P<0·01$) decrease in the blood glucose levels of *P. viridis*. Administration of supra-oesophageal ganglion homogenate into the supra-oesophageal ganglionectomized leeches increased the blood glucose levels significantly ($P<0·05$). Whereas, in the supra-oesophageal ganglionectomized leeches the injections of sub-oesophageal ganglion homogenate produced no effect. Sub-oesophageal ganglionectomy did not produce any significant change in the blood glucose levels even after 24 h of operation. The injection of the supra-oesophageal ganglion homogenate elevated the blood glucose level significantly ($P<0·05$) in the sub-oesophageal ganglionectomized leeches, whereas the injections of sub-oesophageal ganglion did not show any effect.

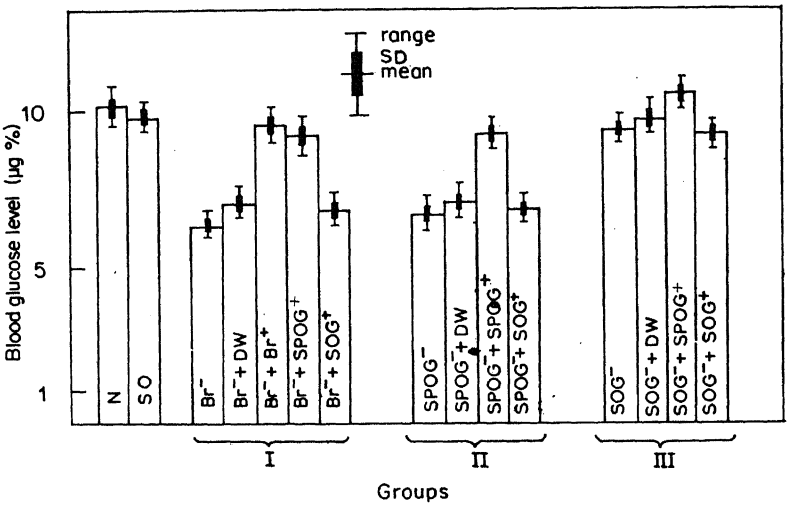

Figure 1. Blood glucose levels in normal and experimental *P. viridis*.
(N), Normal (brain intact); (DW), distilled water; (SO), Sham operated; (Br^-), brain removed; ($SPOG^-$), Supra-oesophageal ganglionectomized (Sub-oesophageal ganglion intact); (SOG^-) Sub-oesophageal ganglionectomized (Supra-oesophageal ganglion intact); (Br^+) Brain homogenate injected; ($SPOG^+$) Supra-oesophageal ganglion homogenate-injected; (SOG^+) Sub-oesophageal ganglion extract injected.

4. Discussion

The blood glucose level in the normal *P. viridis* was found to be 10·2 µg%. Earlier Clark (1964) reported 72–264 mg% of total carbohydrates in the coelomic fluid of a polychaete, *N. hombergi* containing glucose as a major constituent. Hanumante (1975) reported 14·5 mg% of blood glucose concentration in an earthworm, *P. excavatus*. It is obvious that the blood glucose concentration of *P. viridis* is quite low as compared to the concentration of total carbohydrates in *Nephtys* (Clark 1964) and blood glucose in *Perionyx* (Hanumante 1975).

The data of preliminary experiments showed that the injection of the extract of whole brain into 24 h debrained leeches produced a significant increase in blood glucose levels. Since brain is composed of supra-oesophageal and sub-oesophageal ganglia (Bhaskarrao 1983) further experiments were designed to detect which ganglion is involved in the regulation of blood glucose level in *P. viridis*. It was observed that the homogenate of supra-oesophageal ganglia when injected into leeches having their brain, supra- and sub-oesophageal ganglia been removed, increased the blood glucose levels. Injection of sub-oesophageal ganglion extracts had no effects. These results indicate that only the supra-oesophageal ganglionic portion of the brain of *P. viridis* is responsible for increase in the blood glucose level and inferred that supra-oesophageal ganglion is the production site for hyperglycemic hormone in this leech. Similar incidences of neuroendocrine involvement in the control of blood glucose levels have been previously reported for an oligochaete, *Lumbricus terrestris* (Lawrence *et al* 1972) wherein supra-oesophageal ganglion was responsible for the hyperglycemic factor. Interestingly, a contrary finding to Lawrence *et al* (1972) was reported by Hanumante (1975) in another oligochaete worm, *Perionyx excavatus* who observed that the sub-oesophageal ganglion was responsible to increase blood glucose level in that earthworm. No information has come to our notice so far regarding the mechanism and site of hormonal action and the exact responses of the target tissues of leeches to neurohormone(s) which influences the blood glucose levels.

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Effects of ecological factors on the performance of rodenticides in sugar cane

V R PARSHAD, NAFIS AHMAD and G CHOPRA
All India Coordinated Research Project on Rodent Control, Department of Zoology, Punjab Agricultural University, Ludhiana 141 004, India

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Abstract. The sugar cane fields located near river Sutlej at Ladhowal (Lat. 30° 56′N, Long. 75° 52′E) were found infested with 4 rodent species namely, *Bandicota bengalensis*, *Rattus meltada*, *Tatera indica* and *Mus* spp. Of these, *B. bengalensis* was most predominant. Compared to the adjoining wheat, paddy and mung crops the sugar cane fields at growth and maturity stages harboured high rodent population due to frequent waves of rodent immigration as a result of heavy rainfall and flooding of paddy fields in August and September and agronomic operations related to paddy-wheat rotation in most of the fields. Multiple poison baiting of rodents with brodifacoum (0·005%) and zinc phosphide (2·4%) in different fields of sugar cane resulted in significant differences in their performance within and between the fields. With these poisons the rodent control success ranged from 23·0–45·6% in the months of August and September and 68·1–93·4% in October to December. Overall reduction in post-control activity of rodents ranged from 47·2–96·1% in different fields with different poison combinations. The rodent control success seemed to be related to the differences in rodent populations, post-control reinfestation rates, timings of poison baiting, stage of crop development, weather conditions and sowing and harvesting operations in the adjoining crop fields. The environmental factors particularly affected the post-control reinfestation of rodents which as compared to the reference fields (0·35 rodents/100 traps/day) was significantly higher in the treated fields (0·56–2·33 rodents/100 traps/day).

Keywords. Brodifacoum; poison baiting; rainfall; rodents; sugar cane; zinc phosphide.

1. Introduction

Sugar cane suffers particularly from the burrowing, nibbling and feeding behaviour of rodents which cause 10–30% loss in yield as reported in Hawaii (Hood 1968), Guyana (Bates 1969), Mexico (Collado and Ruano 1963) and India (Gupta *et al* 1968). In Florida, Lefebvre *et al* (1978) estimated 7% loss due to rodents in the potential yield of sugar cane. In Punjab, per acre loss due to rodents interms of jaggery has been reported to be 81 kg in standing and 230 kg in lodged sugar cane crop (Bindra and Sagar 1968). High sugar cane damage can be attributed to the readily available source of food and protective cover to the feeding, burrowing, breeding and damaging activities of rodents for almost all the year. The problems of rodent damage and control in sugar cane are particularly severe in tropical and subtropical Asia because here small sized fields of different crops occur simultaneously leading to polyculture cropping at most of the places. Compared to monoculture, the sugar cane in these polycultures is more threatened by the waves of rodent immigration from the surrounding fields as a result of frequent disturbances in these due to ploughing, harvesting and flooding with irrigation or rainfall. For rodent control in sugar cane a number of rodenticides and methods of application have been evaluated and recommended (Hampson 1982). Of these, zinc phosphide baiting continues to be widely used and the recently introduced single dose anticoagulant rodenticides, which showed effective results in other crops (Dubock 1980; Parshad *et al* 1985), have received little attention for control of rodents in sugar cane. Therefore, zinc phosphide and brodifacoum were chosen for the present field trials of rodent control in sugar cane under polyculture crop conditions.

The protection of sugar cane from rodents in polyculture crop areas depends not only upon successful treatment with rodenticides but also on the checking of post-control rodent populations immigrating from the surrounding fields. The post-control reinfestation of rodents in sugar cane and the agronomic and climatic factors of the polyculture area affecting success of rodent control with rodenticides have also been studied.

2. Materials and methods

2.1 *Study area*

Sugar cane fields with a total area of 22·3 ha at 6 distant locations (A–F) were selected at the Central State Farm, Ladhowal, Ludhiana (Lat. 30° 56′N, Long. 75° 52′E). This farm is located on the bank of the river Sutlej and has 1220 ha of crop fields and 180 ha of forest land with natural vegetation. Crops cultivated on this farm include wheat (*Triticum aestivum*), maize (*Zea mays*), masser (*Lens esculentum*), toria (*Brassica campestris*), raya (*B. juncea*) and pea (*Pisum sativum*) in winter, and rice (*Oryza sativa*), maize (*Z. mays*), sugar cane (*Saccharum afficinarum*), mung (*Vigna radiata*) and arhar (*Cajanus cajan*) in summer. The sugar cane fields were surrounded by other crops, mainly the wheat and rice rotations.

Normally sugar cane is planted from mid February to the end of March and harvested mainly from December–February during the subsequent year. Thus, both fresh and ratoon crops of sugar cane provide rodents with an undisturbed shelter almost throughout the year whereas the rodent populations of the surrounding fields are subjected to the pressures of harrowing, sowing, harvesting, flooding during irrigation or rainfall and of other field operations.

2.2 *Poison baits*

An acute toxicant zinc phosphide (80% technical grade powder, Excel Industries Limited, Bombay) and an anticoagulant brodifacoum (0·25% liquid concentrate, Imperial Chemical Industries, England) were used in this study. Zinc phosphide bait (2·4% active ingredient, AI) was formulated on whole millet grains smeared with 1% arachis oil and brodifacoum bait (0·005% AI) was prepared using a mixture of maize flour, wheat flour, sugar and arachis oil (48 : 48 : 2 : 2).

In all the treatments with both the rodenticides, 10–15 g bait were placed on pieces of paper in a checker board manner at 10 m from each other. Zinc phosphide bait was exposed to rodents for one day and brodifacoum for 3–6 days.

2.3 *Treatment schedule*

The sugar cane fields (A–F) were allocated randomly to different treatments of

Table 1. Schedule of poison treatments in 6 (A–F) sugarcane fields.

Field	Age of crop on start (days)	Treatments			Duration (days) between	
		First (T_1)	Second (T_2)	Third (T_3)	T_1–T_2	T_2–T_3
A	145	Br(A)	Br(S)	ZP(N)	43	49
B	149	Br(A)	Br(S)	Br(N)	40	68
C	163	ZP(A)	Br(S)	NT	20	—
D	158	ZP(O)	ZP(N)	Br(D)	49	44
E	206	NT	ZP(O)	ZP(D)	—	35
F	148	R	R	R	—	—

Br, 0·005% brodifacoum; NT, no treatment; R, reference field; ZP, 2·4% zinc phosphide.

Months (A, August; S, September; O, October; N, November; D, December) in which the poison baiting was done are given in parentheses.

poison baiting. Schedule of treatments, age of crop and duration between treatments are given in table 1.

2.4 *Census methods*

Effect of each treatment on rodents was determined by taking the pre- and post-treatment trap indices and census bait consumptions. To calculate the trap index, multi-catch (Wonder) traps were set out in 3 trap lines of 9 traps each with 10 m between the traps. The rodents were trapped for 3 consecutive days after 2 days of prebaiting with wheat grains. Census bait consumption was assessed over 3 days from 27 bait stations (cylindrical tin boxes 6–9 cm in diameter) arranged in the crop fields in the same manner as the traps. The trap index and census bait consumption were taken on different occasions to avoid interaction. The post-treatment census were taken after 3 days of zinc phosphide and 15 days of brodifacoum treatment. The census of rodent populations was also conducted in control fields at times corresponding to those in the treatment fields.

Per cent mortality after all the treatments in each field was calculated from trap indices and the census bait eaten using the following formula (Henderson and Tilton 1955; EPPO 1975).

$$\text{Per cent mortality} = 100\left(1 - \frac{t_2 \times r_1}{t_1 \times r_2}\right), \text{ where}$$

t = census (trap or bait) from treated fields;
r = census from reference fields;
$t_1 r_1$ = pre-treatment trap or bait census;
$t_2 r_2$ = post-treatment trap or bait census.

In order to analyse the effects of different agroclimatic factors on the efficacy of each rodenticide treatment, per cent change between pre- and post-treatment trap and bait census has been calculated.

2.5 *Meteorological data*

Information regarding the daily rainfall, humidity and temperature recorded by the Department of Agrometeorology, Punjab Agricultural University, Ludhiana has been used.

2.6 *Population changes*

The differences between the post-treatment census and the subsequent census (i.e. pre-treatment census of the next treatment) formed the basis for determination of rodent reinfestation rate (rodents/100 traps/day) during the period between two treatments. To compare the levels of rodent populations between sugar cane and the adjoining crop fields, a trap census was taken from the latter at least once during the crop season. Records of sowing and harvesting and other related field operations in the adjoining fields were also kept to analyse their possible impact on rodent populations in sugar cane.

3. Results and discussion

3.1 *Field efficacy of rodenticides*

The poison baiting of rodents with 0·005% brodifacoum and 2·4% zinc phosphide in sugar cane fields resulted in significant differences in their efficacy within and between the fields (table 2, figure 1). Irrespective of the poison, the treatments were less effective during rainy months of August and September (figure 2) than during October to December. Rodenticidal treatments resulted in only 22·4–45·6% control success with brodifacoum in fields A–C in August and September and 23% with zinc phosphide in field C in August. In contrast, brodifacoum baiting in November and December resulted in 68·1% and 78·9% control success in fields B and D. Similarly, 73·4–93·4% control success was achieved with zinc phosphide baitings during October–

Table 2. Efficacy of rodenticidal treatments and post-control reinfestation of rodents in sugarcane fields.

Field	Reduction (%)[a]			Cumulative effect of treatments (% mortality)[a]	Reinfestation rate rodents/100 traps/day	
	T_1	T_2	T_3		T_1–T_2	T_2–T_3
A	45·6	26·1	73·4	72·9	1·46	—
B	30·6	22·4	68·1	47·2	2·33	—
C	23·0	24·8	—	56·1	—	—
D	84·1	85·3	78·9	91·5	1·47	0·56
E	—	90·6	93·4	96·5	—	1·68
F[b]	+39·6	+22·6	+31·1	—	0·35	

T_1, T_2, T_3 are first, second and third treatments as scheduled in table 1.

[a] Figures are the mean of 2 census methods i.e. trap and bait census.

[b] +, sign indicates increase in rodent population and figure of 0·35 represents overall reinfestation rate.

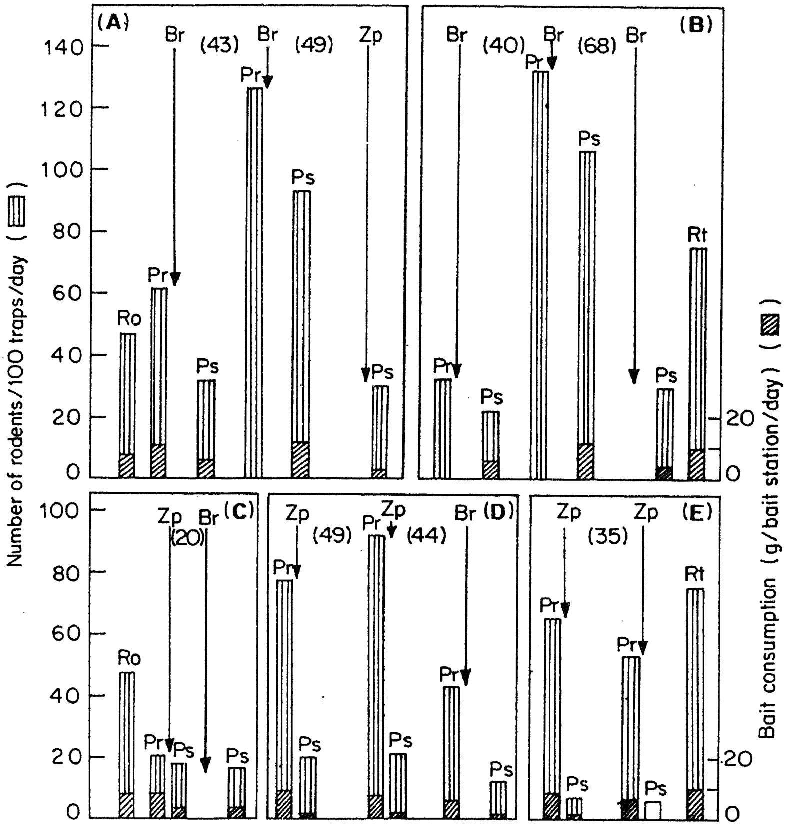

Figure 1. Effects of rodenticidal treatments on the trappable population and census baiting of rodents in sugar cane fields. The vertical arrows indicate the treatments. Figures in parentheses are the number of days between the treatments. The lag period between the poison treatment and post-treatment census was 3 days with zinc phosphide and 15 days with brodifacoum. (Br, brodifacoum; R_o, initial census in the reference field; R_t, final census in the reference field; Pr, Pre-treatment census; Ps, post-treatment census; Zp, Zinc phosphide).

December. Poison baits containing brodifacoum (Dubock 1980; Parshad *et al* 1985) and zinc phosphide (Fitzwater and Prakash 1973) have been shown to possess uniformly high toxicity to several species of tropical and sub-tropical rodents. The time and treatment related differences in their performance against rodents in sugar cane could not be attributed to their toxicity but to some ecological and behavioural factors.

3.2 *Differences in rodent populations*

The trapping of rodents from the experimental fields (A–F) showed a common occurrence of *Bandicota bengalensis*, *Rattus meltada*, *Tatera indica* and *Mus* spp. in sugar cane. Their relative abundance varied in different fields (figure 3). In fields B and D, *R. meltada* had the higher trappable population while in others *B. bengalensis*

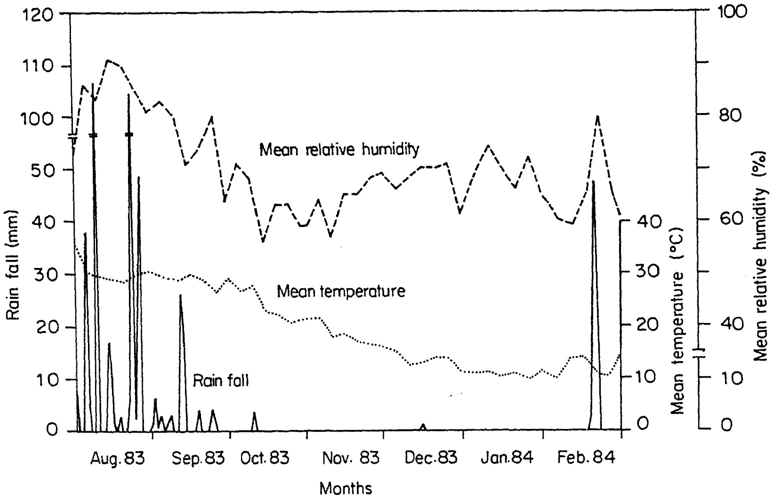

Figure 2. Changes in rainfall, humidity and temperature during the course of field experiments of rodent control in sugar cane.

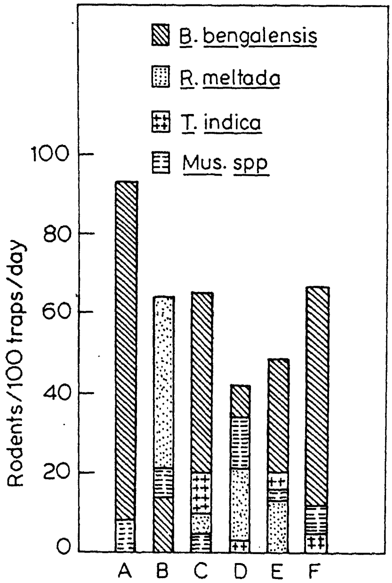

Figure 3. Relative abundance of different species of rodents in experimental fields of sugar cane.

dominated. These differences in the rodent populations appeared to be related to the different locations of the sugar cane fields and to the rodent harbourage of the surrounding cropped and non-cropped areas. How these differences in rodent populations affected the relative performance of rodenticides in sugar cane fields is not clear. One of the probable factor which could be considered to have affected the performance of rodenticidal treatments was the pre-control rodent populations (figure 1). Heavy rainfall in August (figure 2) and frequent flooding of paddy fields resulted in an astonishing increase in rodent populations in sugar cane fields A and B which showed 123·6 and 132·5 rodents/100 traps/day trap indices respectively. Here, a single brodifacoum treatment in September reduced the trap index by 26·6 and 22·4% respectively. Low control success was also achieved when fields had low pre-control population as in field C (figure 1). In contrast, 68·1% and 78·9% control success occurred with brodifacoum when pre-control trap indices were 106 and 43 rodents/100 traps/day. A negative correlation between pre-control trap index and per cent reduction in rodent activity was evident with zinc phosphide treatments made during the dry weather from October–December (table 3).

Though other factors might be important but the data presented here clearly indicated the effect of pre-control population numbers on the performance of rodenticidal treatments in sugar cane crop.

3.3 *Post-control reinfestation of rodents*

The duration between the treatments varied from 35–68 days in different fields, except 20 days in field C (table 1). During the gap of 35–68 days between the treatments rodents were found to reinfest the cane fields (figure 1). The rate of reinfestation varied in different fields and was minimum in the reference (table 2). The post-control reinfestation of rodents occurred due to weather conditions, breeding of rodents and sowing and harvesting operations in adjoining crop fields as described in the following section.

3.4 *Effects of other crops*

Differences in the trappability of rodents in sugar cane at its different stages and the adjoining crop fields are given in table 4. At the early growth stage in March the sugar cane fields had a few rodent burrows at the edges and the trappability was minimum than the subsequent growth and maturity stages. In this region wheat is harvested in April–May and most of these fields are prepared for paddy transplantation

Table 3. Relationship between pre-control trap index and per cent reduction in rodents with zinc phosphide treatments.

Pre-control trap index rodents/ 100 traps/day	Reduction in rodents (%)
55·0	93·4
66·5	90·6
79·0	84·1
91·0	73·4
91·5	85·3

The value of correlation coefficient, $r = -0{\cdot}83$ (calculated according to Sokal and Rohlf 1973).

Table 4. Comparison of rodent trappability (rodents/100 traps/day) in sugarcane and its surrounding crop fields.

Crop	Month	Stage of crop development	Total trap index Mean (range)	Trap indices of different species			
				B. bengalensis	*R. meltada*	*T. indica*	*Mus* spp.
Sugarcane	March	30–60 days post-harvest	8·64 (0–14·88)	6·17	1·47	—	—
	August	Late growth	40·75 (22·2–60·8)[a]	25·25	8·53	3·22	3·75
	September–October	Maturing	101·50 (71·56–132·53)[a]	52·38	20·25	10·75	18·13
	November–December	Mature	87·93 (67·0–106·31)	48·93	14·33	11·67	13·0
Wheat	March	Maturing	20·37 (18·52–22·22)	11·11	3·70	3·70	1·85
Paddy	September	Maturing	45·68 (37·04–62·97)	28·40	8·64	1·27	7·41
Mung	September	Maturing	27·27[b]	4·55	18·18	0·0	4·55

[a] Rodents in these fields were treated with rodenticides (table 2) but here the post-control reinfestation enhanced the trappability.

[b] Based on trapping from one field only. Figures of other crops are the mean and range of trappings from 3 or 4 fields.

in June–July. Emigration of rodents from these fields to sugar cane combined with breeding (Bindra and Sagar 1975; Sood and Ubi 1975) seemed to enhance 5 fold trappability of rodents in August than in March (table 4). Heavy rainfall in August and September (figure 2) and frequent irrigation of paddy fields during this period led to further increase in rodent population in sugar cane. Despite the post-control reduction in rodents in fields treated in August–September (table 2), the high trappability (87·93 rodents/100 traps/day) in November and December was related to harvest of paddy in October.

In nature several factors may effect the dispersal, migration and density of rodents in a given area (Lidicker 1975). From the present study, it is difficult to predict the nature and process of rodent migration but it appears that changes in the population levels in sugar cane relate to the disturbed conditions in the vicinity and vice-versa.

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Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros*

R JEYARAJ, T THANGARAJ, C A VASUKI and M ARUCHAMI
Department of Zoology, Kongunadu Arts and Science College, Coimbatore 641 029, India

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Abstract. Characteristics of cuticular phenoloxidases of the coconut pest, *Oryctes rhinoceros* were studied biochemically with respect to substrate specificity, pH optima, thermostability and the behaviour of the enzyme in the presence of various chemicals. Biochemical studies indicate that the larval cuticle contains tyrosinase (EC, 1·10·3·1 Ortho diphenol: O_2 oxidoreductase), and laccase type of enzymes (EC, 1·10·3·2. Para diphenol: O_2 oxidoreductase). These enzymes showed similarity to enzyme types A and C with respect to the enzyme activity in the larval cuticle of *Calliphora vicina*. In the electrophoretic study two fractions are discernable in the gel which confirms the occurrence of two enzymes namely tyrosinase and laccase types.

Keywords. Phenoloxidases; *Oryctes rhinoceros*; tyrosinase; laccase.

1. Introduction

The insect phenoloxidases are known to play important roles in cuticular sclerotization and defensive functions (Andersen 1979; Brunet 1980). Subsequent to the report of Dewitz (1902a, b) in the blow fly, various workers have studied the nature and mode of sclerotization in insects. Two enzymes namely tyrosinase and laccase types of enzymes have been reported in insects. The enzyme tyrosinase is reported to occur both in the cuticle and blood, whereas, the latter is found to occur in the cuticle alone.

The enzyme associated with defensive function is said to be soluble whereas, the cuticle sclerotizing enzyme is reported to occur as soluble and insoluble forms. Yamazaki (1969, 1972) has reported that presclerotized pupal cuticle of *Bombyx mori* and *Drosophila* contain an insoluble enzyme and could be solubilised by trypsin digestion. Subsequently, Andersen (1978) has purified the trypsin soluble phenoloxidase in the cuticle of desert locust, *Schistocerca gregaria* and the enzyme was found to oxidise both ortho and paraphenols. Further studies in the above animal have revealed that the cuticle contains an insoluble enzyme which also activate the side chain of N-acetyl dopamine (NADA). However, it is not resolved yet that these two enzymes are one and the same entity (Barrett and Andersen 1981). Soluble phenoloxidases have been separated and purified in the larval cuticle of *Calliphora vicina* by Barett and Andersen (1981) and are reported to be involved in wound healing (tyrosinase type A) and cuticular stabilization (laccase types B and C). Recently, Barrett (1984) has reported that soluble enzyme, similar to that of enzyme type A in *Calliphora vicina* is known to occur in the cuticle of *Calpodes ethlius* and named it as wound healing phenoloxidase. In this paper, we report the characteristics of soluble enzyme(s) from the cuticle of coconut pest, *Oryctes rhinoceros*.

2. Materials and methods

Third instar larvae of *O. rhinoceros* (5 cm-length) were collected from the manure pits in the garden nearer to the college campus and were reared in plastic troughs (capacity 5 liters) by providing cow dung as the source of food. For the preparation of enzyme, larval cuticles were removed carefully and washed several times in distilled water and the adhering tissues were removed. Thoroughly cleaned cuticles were observed under microscope for the adhering tissues and blood cells if any. Cuticles were cut into small pieces and ground well using morter and pestle in ice cold borate buffer 0·003 M (0·1%) pH 9·0. The crude enzyme was separated after centrifugation at 5000 rpm for 20 min. The enzyme was precipitated by ammonium sulphate (50%) and dialysed against the phosphate buffer (pH 7·0) extensively for 48 h at 10°C. The flocculent materials were removed and the clear solution was used as the enzyme source.

Phenoloxidase activity was measured following the method of Andersen (1980), with little modifications. 100 μl of the enzyme was incubated with 100 μl of 0·01 M substrate dissolved in 0·01 M phosphate buffer (pH 7·0) (the pH of the incubation mixture was 7·2). After 15 min at 30°C 1·8 ml of (0·01 M) phosphate buffer (pH 7·0) was added and the intensity of colour developed was measured at 480 nm against the blank using Spectronic 21-spectrophotometer (Bausch–Lomb). Protein content was measured following the method of Lowry *et al* (1951). The enzyme was separated electrophoretically following the method of Jolley and Mason (1965) and were localised by incubating the gels in suitable substrates (0·01 M). (4-methyl catechol, Dopamine, Dopa, Hydroquinone) for a period of 20 h and the bands were visualized.

3. Results and discussion

The enzymes separated from the cuticle of *O. rhinoceros* showed activity towards the following substrates in the order of Dopamine 4-methyl Catechol methylhydroquinone hydroquinone Dopa tyramine (table 1). The enzyme showed activity towards O-

Table 1. Activity of enzyme, phenoloxidase on various substrates.

Substrates	Enzyme activity (absorbance/mg protein/min)
Monophenols	
Tyrosine	0·0
Tyramine	0·02
Diphenols	
4-methyl catechol	0·13
Methyl hydroquinone	0·11
Hydroquinone	0·10
Dopa	0·04
Dopamine	2·0
Protocatechuic acid	0·0
Resorcinol	0·0

Each value represents average of 3 different determinations.

diphenol and paradiphenol as well as monophenols suggesting thereby that both tyrosinase and laccase type of enzymes are present in the cuticle of *O. rhinoceros*. However, the enzyme showed inability to oxidize the protocatechuic acid. Similar observation has also been reported in the case of Calliphora larval cuticle, in which 3 types of enzymes have been separated (enzyme types A, B and C). Enzyme A is designated as tyrosinase and it is suggested that it may perform the function of wound healing whereas, B and C are of laccase types and are involved in cuticular sclerotization.

Since the enzyme obtained from the cuticle after ammonium sulphate precipitation, it is not possible to distinguish at this level whether the cuticle contains more than one enzyme. Recently Barrett (1984) has reported that the cuticle of *Calpodes ethlius* is known to contain only the enzyme A type which showed similar properties to that of the enzyme A type in the *Calliphora erythrocephala.*

The cuticular enzyme in the *O. rhinoceros* showed similarity to the enzyme type A as in the case of *C. vicina* (Barrett and Andersen 1981) and *C. ethlius* (Barrett 1984). All these enzymes are sensitive to phenylthiourea, sodium diethyl dithiocarbamate and are unstable above 60°C. These enzymes showed pH optimum around 6·5–7·2 (tables 2, 3 and 4).

Electrophoretically it has been identified that there are two fractions in 7% gel. One fraction was discernable at the top of the gel, whereas another fraction is just below the first fraction (figure 1).

It has been suggested that the enzyme tyrosinase occurring in the cuticle as proenzyme is performing the function of wound healing. However, a detailed study on this aspect is wanting. The enzyme laccase is reported to be involved in the cuticular sclerotization. Andersen and Roepstorf (1982) have suggested that cuticular sclerotization is mediated by two ways that N-acetyl dopamine is oxidised to quinone by the enzyme phenoloxidase and the same compound can also be oxidized in side chain of NADA giving rise to unsaturated NADA by desaturase which can further be oxidised to unsaturated quinone by the diphenol oxidase. Based on these findings they have suggested that saturated quinone involving pathway is leading to the formation of coloured cuticle whereas, the unsaturated quinone forming pathway

Table 2. Effect of various chemicals in the activity of the enzyme, phenoloxidase.

Chemicals	Concentration (mg/ml)	Activity (absorbance/mg protein/min)	Inhibition (%)
Without treatment	0·0	2·0	0·0
Phenylthiourea	1·0	0·001	96·3
	0·5	0·002	92·6
Thiourea	1·0	0·013	51·86
	0·5	0·013	51·86
Sodium fluoride	1·0	0·019	29·63
	0·5	0·018	33·34
Sodium azide	1·0	0·015	44·45
	0·5	0·016	40·75
Diethyldithio carbamate	1·0	0·003	88·89
	0·5	0·004	85·19

Each value represents average of 3 different determinations.

Table 3. Effect of temperature on the stability (absorbance/mg protein/30 min) and activity of the enzyme, phenoloxidase.

Temperature (0°C)	Activity recovered after treatment with various temperatures	Activity
20	0·060	0·069
30	0·164	0·170
40	0·188	0·178
50	0·220	0·270
60	0·234	0·310
70	0·040	0·050
80	0·040	0·010

Each value represents average of 3 different determinations.

Table 4. Effect of pH on the activity of enzyme, phenoloxidase.

pH	Activity (absorbance/mg protein/30 min)
5·5	0·090
6·0	0·100
6·5	0·155
7·0	0·175
7·5	0·140
8·0	0·100
8·5	0·080

Each value represents average of 3 different determinations.

leads to colourless cuticle. In this context it is of interest to mention that *O. rhinoceros* is having the white cuticle in the larval stage while, it is turned to brownish red during pupal and black colour in the adult stage. It may be suggested that the sclerotization of larval cuticle may be involved by producing the unsaturated quinone involving the phenoloxidase and desaturase in the larval stage.

It has been suggested that the sclerotization of white cuticle is mediated by the formation of quinone methide rather than the formation of unsaturated NADA involving the enzymes phenoloxidase and desaturase (Sugumaran and Lipke 1983). This alternative scheme justifies the formation of catechol derivatives, tritium release from β-position and catechol dimerization by the formation of quinone methides. Since NADA is generally accepted as the unique sclerotising agent, it has been suggested the possibility of the synthesis of tautomeric quinone methide and the quinone from NADA. However, formation of quinone methide from NADA becomes more complicated than the substrates containing methylene group at 4th position (Sugumaran and Lipke 1983). It is relevant to mention that the phenoloxidase oxidases strongly 4 methyl catechol and methyl hydroquinone comparatively equivalent to Dopamine as has been observed in the present study. Similar observation has also

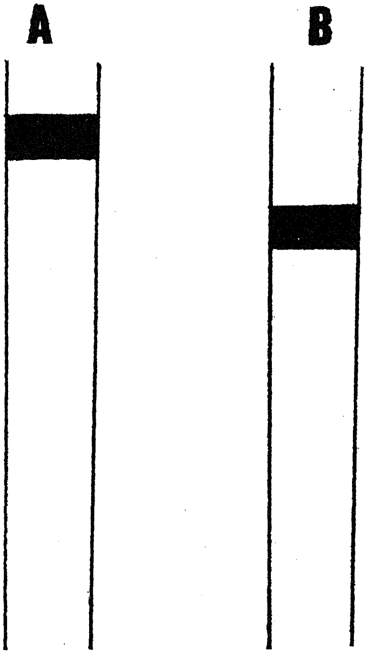

Figure 1. Localization of phenoloxidases in 7% polyacrylamide gel. **A.** Tyrosinase. **B.** Laccase.

been reported in the case of *B. mori* (Yamazaki 1972) in the locust cuticle *S. gregaria* (Andersen 1978).

The electrophoretic study revealed that there are atleast 2 enzymes occurring in the larval cuticle of *O. rhinoceros*. A detailed study is underway to elucidate the various properties of these enzymes.

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Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants

R S ANNADURAI and R VELAYUDHAN
Entomology Research Institute, Loyola College, Madras 600 034, India

MS received 30 June 1986

Abstract. The vector role of thrips in pollinating several fabaceous flowers has been studied. The flowering phenology showed a coincidence with the life cycle of the thrips species. The population dynamics of the thrips, crop-weed dispersal, the pollen carrying efficiency and the percentage of pod set are discussed.

Keywords. Thrips; pollination; pollen load; pod set.

1. Introduction

Shaw (1914) confirmed the vector role of thrips in pollination on Sugarbeet and Annand (1926) on *Cucumis melo*. Subsequent information relates to reports by Billes (1941) on *Cacao*, Mathur and Mohan Ram (1978) on *Lantana camara*, Syed (1978) on oilpalm and Rust (1980) on *Arisaema triphyllum*. Thrips-flower association in the members of Compositae has been extensively worked out in terms of species composition, species packing, ecological succession, pollinator dispersal, pollen-stigma interaction etc (Ananthakrishnan *et al* 1981; Gopinathan *et al* 1981). Information on the role of thrips as pollinators of fabaceae appears very meagre, except for that on *Phaseolus lunatus* by Mackie and Smith (1935); on *Cajanus cajan* by Williams (1977) and on *Dolichos lablab* by Velayudhan *et al* (1985). Pollination by thrips and the production of viable seeds in the members of Solanaceae have been correlated with the degree of Stigmatic receptivity (Velayudhan and Annadurai 1986). The role of thrips in the pollination of some fabaceous plants such as *Cajanus cajan* (L), *Vigna catjang* Walp., *Phaseolus mongo* L., *Phaseolus vulgaris* L., *Arachis hypogaea* Willd., *Crotalaria* sp, *Tephrosia purpurea* Pers., *Sesbania aegyptiaca* Pers. and *Pongamia glabra* Vent. is discussed in this investigation.

2. Materials and methods

Random weekly collections of thrips were made from 20 flowers during its period/season of occurrence in order to record not only the fluctuations in the populations, but also to observe the pollen load by counting the number of pollen grains attached to the various parts of the body. Dispersal of thrips from flower to flower was observed by tagging them with gilt powder. In order to assess the role of thrips in the transfer of pollen from flower to flower in an inflorescence, emasculation technique was carried out by removing the anthers in all the buds in the upper part of the inflorescence thereby preventing self fertilisation, at the same time covering the complete inflorescence with a plastic bag, so as to prevent other agents from pollinating the flower, thus allowing only thrips to do the role. A comparative assessment of the efficiency of pollination by various agents was estimated in terms of pod setting. This was investigated by 3 sets of experiments (i) hand pollination, (ii) pollination by thrips alone and (iii) natural condition where all the other foraging insects played their role. Aspects regarding the biology of thrips was studied by keeping cultures of thrips in flowers/inflorescence within glass chimneys dipped in knop's solution. Scanning electron microscopic studies to depict the mode of attachment of pollen grains on the various parts of the body were made with etherised specimens carrying pollen grains and dried in a critical point drier. The specimens were later fixed on an aluminium stub using double adhesive tape and was coated with gold for 3 min in an ion coater. Micrographs were taken using Hitachi scanning electron microscope (Model S 415 A) under 15 KV emission current.

3. Observations and results

The availability of the various fabaceous plants referred to above, their flowering periodicity and the period of maximum population is indicated in figure 1. Among the various crops plants taken for the present study *Arachis hypogaea*, *Phaseolus mongo*, *Phaseolus vulgaris*, *Vigna catjang* and *Cajanus cajan* are seasonal and flowering in these plants is also restricted to a period ranging from 40–60 days. Perennial plants as *Tephrosia purpurea*, *Sesbania aegyptiaca*, *Pongamia glabra* also show seasonality in blooming and only in the case of *Crotalaria* the flowering was observed throughout the year.

Megalurothrips distalis Karny, *Frankliniella schultzei* Trybom are the two species

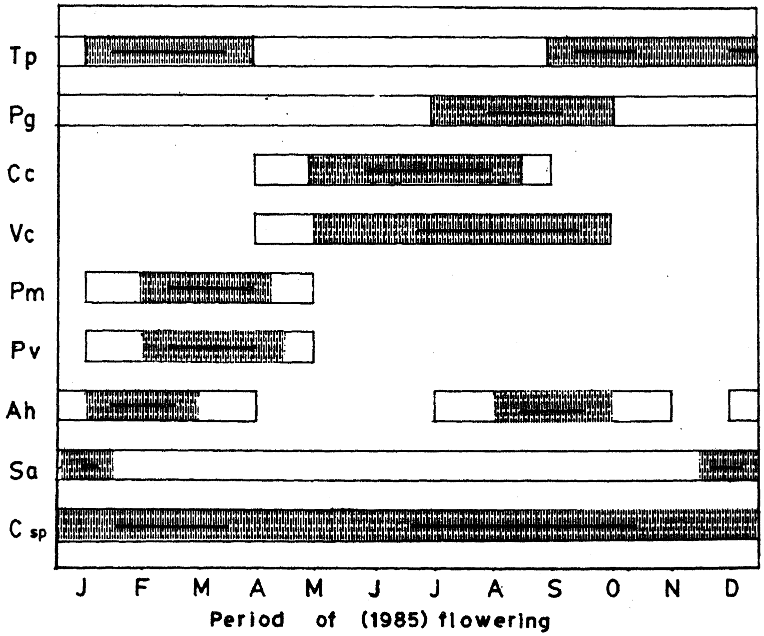

Figure 1. Availability of various Fabaceous plants during the course of the year, the flowering periodicity (shaded area), and the line represents the peak population period.

occurring in all the flowers studied with *Haplothrips gowdeyi* (Franklin) at a comparatively lower frequency in the flowers of *Cajanus cajan* and *Vigna catjang*. Synchronisation of the flowering periodicity and the life cycle of thrips with the flowering phenology was evident. The continuous availability of anyone of these flowers in and around the vicinity of the crop fields is attributable to the continued sustainance of the thrips population. The population dynamics of both *M. distalis* and *F. schultzei* as well as the pollen load of the larvae and adults is indicated in figure 2. *M. distalis* and *F. schultzei* occurred continuously during the flowering period, the population of *M. distalis* being dominant over *F. schultzei*. A comparative study of the population of *M. distalis* and *F. schultzei* among the various fabaceous flowers showed a clear dominance of both the larvae and adults of the former species. In the case of *Pongamia glabra* the maximum number of *M. distalis* larvae was 150 individuals/20 flowers whereas the number of adults was 95 individuals/20 flowers. The population of larval *F. schultzei* in the flowers of *P. glabra* registered 30 individuals/20 flowers and 20 adults/20 flowers.

In the case of *Sesbania aegyptiaca* the population of *M. distalis* and *F. schultzei* recorded the least number as compared to the other host plants. The continuous availability of such perennial host plants as *S. aegyptiaca*, *T. purpurea*, *Crotalaria* sp. and *P. glabra* within the same vicinity where the crop plants viz *C. cajan*, *V. catjang*, *P. vulgaris*, *P. mongo* and *A. hypogaea* are cultivated positively prevented the complete

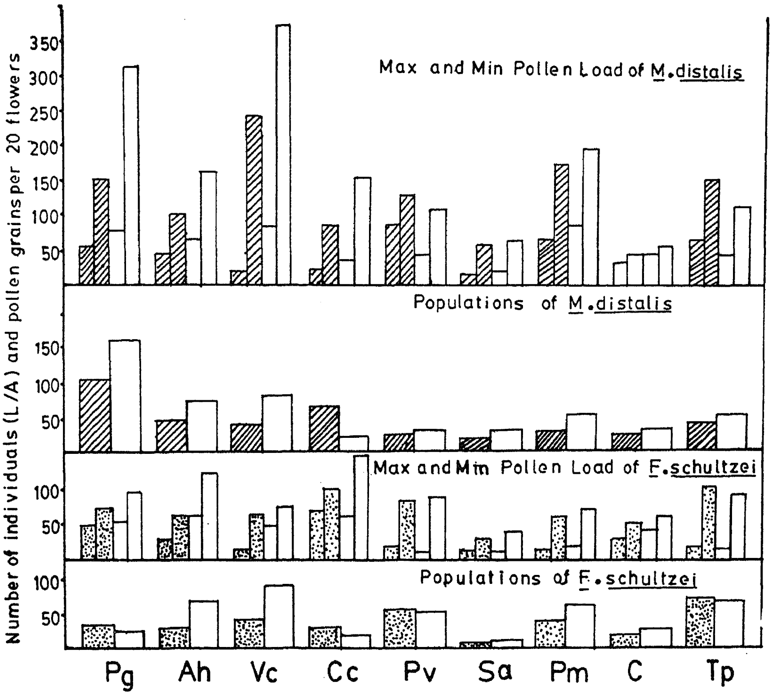

Figure 2. Average maximum and minimum pollen load of the adults (shaded) and larvae correlated with the mean population of the two thrips species within the flowers of fabaceous plants.

(Tp, *T. purpurea*; Cc, *C. cajan*; Pm, *P. mongo*; Ah, *A. hypogaea*; C, *Crotalaria* sp.; Pg, *P. glabra*; Vc, *V. catjang*; Pv, *P. vulgaris*; Sa, *S. aegyptiaca*).

decline of the thrips population, at the time of non-availability of the flowers. The pollen carrying efficiency of *M. distalis* larvae was maximum in the case of *V. catjang* ranging from 19–95/individuals. But *F. schultzei* larvae and adults showed a pollen load of 5–18 and 4–14/individuals respectively. In all the flowers studied the pollen carrying efficiency was higher in the case of *M. distalis* larvae.

The papilionaceous flowers by its special type of flower structure form a suitable micro-environment for foraging, feeding, breeding and oviposition site. The life cycle duration of both *M. distalis* and *F. schultzei* was around 12–18 days, the initial infestation occurring between the third and fifth day of the bud stage, with the adults feeding on fleshy regions of the standard and keel petal resulting in the development of numerous necrosed spots within the bud. Oviposition sites were mainly around the keel petals and after an incubation period of 4–5 days, the larval emergence coincided with the maximum amount of floral nectar the feeding being mainly on nectar and pollen. The scanning electron micrographs presented in figure 3 indicate the exact mode of pollen attachment to the various body parts such as abdominal segments, wing fringes, antennae, legs etc. The architecture of the pollen grains coupled with the setal arrangement aid in the attachment of the pollen grains to the various parts of the body. The results of various experiments viz hand pollination, pollination by thrips alone and natural pollination in terms of percentage of pod set presented in table 1 showed that the pod setting was comparatively higher in the case of pollination by thrips alone (80%) than hand pollination (60%) and comparatively lesser than natural pollination (90%) which indirectly shows that thrips do play some role in the pollination of these fabaceous flowers.

4. Discussion

The phenology of both the flowers and the colonising thrips appear important in the proper understanding of not only the population dynamics of the concerned pollen vector, but also the pollination mechanism involved in the thrips-flower association. Species composition and species dominance were clearly evident in all the fabaceous flowers studied, the dominant species being *Megalurothrips distalis*. The ability of thrips to carry pollen grains from the anther to the stigma of the same flower or another flower depends on the size, viscocity, exine architecture of the pollen grains, attractiveness of the flower as well as the population, diversity, number of body setae and body surface texture of the thrips species present. Thrips infesting the flowers at the bud stage effect an early pollination. Thrips movement inside the keel petals enable the transfer of pollen to the receptive surface of the capitate stigma. Frequent movement of the adult as well as the larvae inside the keel petals exerts substantial stress on the anther walls when it passes over the lobes enhancing the anther dehiscence. Velayudhan *et al* (1985) have reported that during the nonflowering season of *D. lablab* thrips are forced to migrate to *P. glabra* and other weeds in the same locality which incidentally flowers during that period, thus enabling the species to sustain their population during the off-season of the crop. The present study also revealed that perennial plants such as *P. glabra*, *S. aegyptiaea*, *Crotalaria* sp., and *T. purpurea* serve as reservoirs of the thrips species during the off season of the crop plants such as *V. catjang*, *C. cajan*, *A. hypogaea*, *P. mongo* and *P. vulgaris*.

Hammons and Leuk (1966) have reported that larvae inside the keel petals feeding on the nectar and pollen grains accidentally resulted in the self pollination of *Arachis hypogaea* flowers. Sen and Sur (1964) have reported that *Taeniothrips distalis* visit

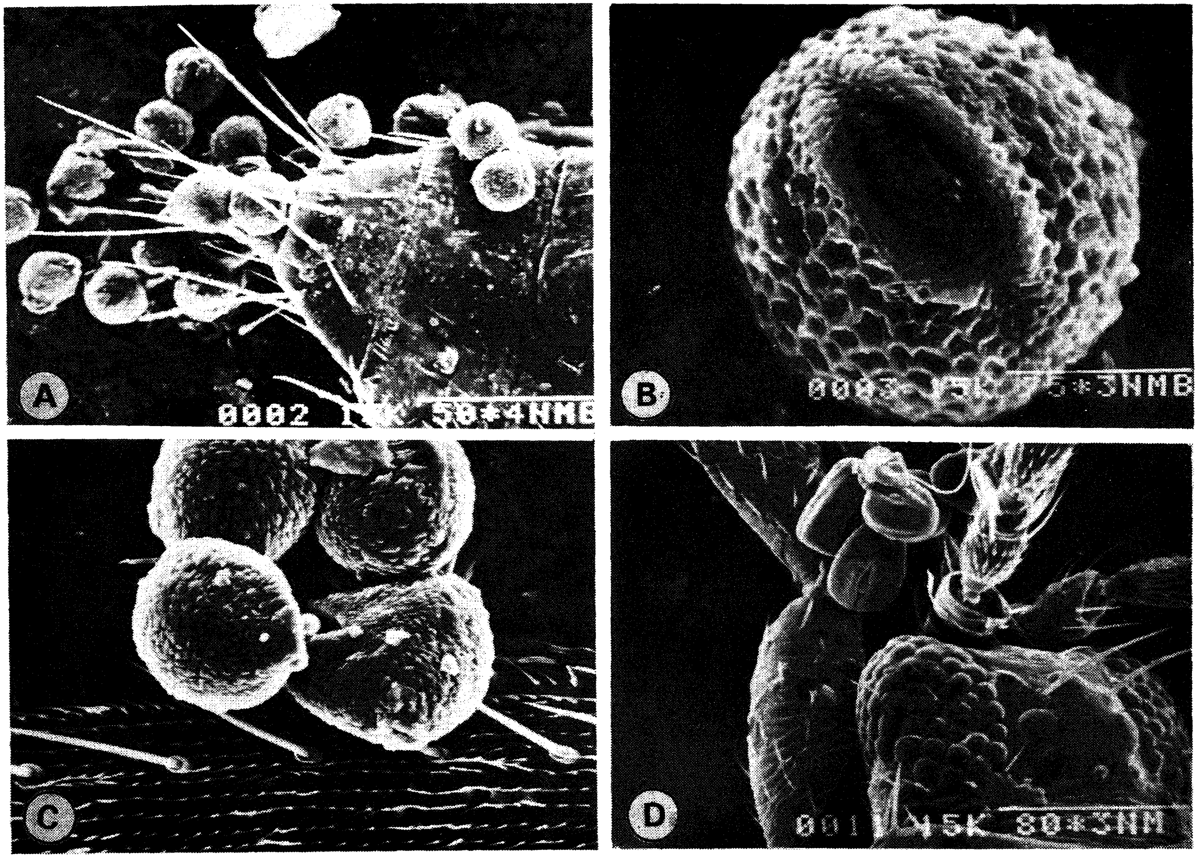

Figure 3. **A.** Pollen grains of *C. cajan* attached to the last abdominal segment of *M. distalis*. **B.** A single pollen grain of *C. cajan* enlarged. **C.** Pollen grains of *C. cajan* attached on the wing setae. **D.** Pollen grains of *Arachis hypogaea* attached to the setae on the antennae and forelegs.

Table 1. Percentage of pod set on various fabaceous plants.

Host plants	Hand pollination	Natural pollination	By thrips
T. purpurea	56·1 ± 8·62	89·2 ± 5·80	79·8 ± 8·79
C. cajan	61·2 ± 2·49	91·2 ± 6·52	80·4 ± 4·92
V. catjang	61·1 ± 7·72	90·3 ± 6·97	80·6 ± 5·19
A. hypogaea	58·9 ± 4·46	88·7 ± 7·36	80·4 ± 4·85
S. aegyptica	59·6 ± 4·71	89·6 ± 5·44	81·1 ± 3·72
P. vulgaris	60·7 ± 4·79	94·4 ± 3·61	78·6 ± 5·79
P. mongo	61·1 ± 5·70	91·6 ± 6·34	79·9 ± 5·53
Crotalaria sp.	58·1 ± 5·69	91·7 ± 5·33	81·3 ± 5·25
P. glabra	56·1 ± 6·08	87·6 ± 4·86	76·2 ± 4·28

Values represent mean of 10 replicates.
Mean ± SD.

pigeon pea flowers. William (1977) has found thrips were numerous in pigeon pea buds and flowers and they entered the buds that had began to open. But a clear understanding of their effective role in pollinating several other fabaceous flowers is evident in this study. However, the importance of thrips in self pollination, or cross pollination of the fabaceous flower is difficult to ascertain but their relative abundance and pollen load may compensate for their small size in contrast to other pollinating agents.

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Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device

PRAKASH NAUTIYAL, A S SAKLANI and H R SINGH
Pollution Monitoring Lab, Department of Zoology, University of Garhwal, Srinagar 246 174, India

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Abstract. A technique to sample plankton efficiently in the fluvial systems is being propounded along with a note on the need and design of the plankton sampling device suited to torrential reaches. The technique, christened as discharge-time method, has certain advantages. The requirements are minimum (detachable bucket of the conical plankton net with bolting silk cloth of required mesh size) and the efforts are nominal. In order to sample plankton the device has to be dipped into the river with its open end facing the current, for a pre-calculated period of time. The formula, 't' seconds $= x/4v$ was computed to calculate the time for which the device should remain submerged. It is thus, an efficient and time saving technique for large volumes can be filtered within seconds. The validity of this method has been assessed by employing 't' test.

Keywords. Fluvial system; plankton; sampling; torrential reaches; discharge-time method.

1. Introduction

Water sampling in the fluvial systems, especially in the torrents is not without limitations. In the alluvial plains where long stretches are navigable, sampling is facilitated by country made vessels. Contrary to it difficulties of many kind appear while sampling in the torrential stretches. The latter are characterised by high gradient, deep gorges which account for alternating rapids and pools as well as high velocity and turbulence of water.

The present paper deals exclusively with plankton sampling in the fluvial systems, especially the torrential waters. Various types of plankton nets ranging from simple conical Hansen, Juday, Wisconsin, Birge Cone and Clarke Bumpus nets have been described alongwith their merits and demerits by Welch (1948), APHA, AWWA, WPCF (1975) and Trivedy and Goel (1984). In India while limnologists may use them, depending on the nature of water body to be explored and efficiency desired, the riverine ecologists, according to the literature available, are still short of an adequate technique. Chakrabarty *et al* (1959), while conducting studies on the hydrobiology of the river Yamuna at Allahabad, have sampled plankton by collecting and filtering the river water. Lakshminarayana (1965) used a country boat and a conical plankton net with 50 ml clean and rimmed pyrex tube attached to its caudal end. The vessel, according to him, moves against the current and the net is lowered into the river for 5 min. Similarly, John (1976) while sampling plankton from the river Kallayi (Kerala) made use of a canoe, the speed of which was kept constant and a plankton net having 77 threads/cm was towed for 10 min.

Evidently, no uniform technique exists as far as plankton sampling in the fluvial system is concerned. If the task of plankton enumeration has to be achieved by the formula:

$$n\,(\text{units/litre}) = \frac{(a \times 1000)\,c}{1}\ \text{(Welch 1948)},$$

a known quantity of water '*l*' has to be filtered from which a concentrate '*c*' has to be prepared before the average number of plankton '*a*' and finally the number of plankters/litre '*n*' can be obtained. The quantity of water filtered is thus of prime importance, irrespective of the means sought to achieve this goal. In usual practice containers of known volume are used to collect water, which in itself is a tedious and time consuming process. Further, strong current and turbulence in the torrential stretches, prevents navigation and mid-stream sampling, thus rendering the sample inadequate. However, curvilinear path and thorough mixing of water may act as an approximate compensation for samples collected along the river banks.

The above account discusses the major obstacles encountered while sampling plankton in the torrential waters. They can be overcome if an efficient method of filtering the desired volume of river water and a way to obtain an adequate sample at a distance of at least 1·5 meters from the river bank, can be devised. Idea of propounding a new and efficient technique, was thus conceived. The same has been christened as Discharge–Time Method. It is based on the experiments conducted at Srinagar–Garhwal, through which flows the turbulent Alaknanda (altitude 500 msl; latitude 30°11′), a major tributary of the Ganga.

2. Result and discussion

2.1 *Discharge–time method*

The technique involves the recording of velocity with which the water flows at the sampling site, calculating the discharge through the plankton net's detachable bucket (referred to hereafter as bucket only in the text) and converting the value into time for which the device should remain submerged so as to allow the desired volume of the water to be filtered. The requirements are nominal; the bucket of a simple conical plankton net (with bolting silk of desired mesh size, figure 1) provided with 1·5 meter long handle (a modification, discussed later in the text), a current meter or a suitable float and a stop watch. The method can be successfully implemented in two steps, prior to which a formula has to be computed, equipped with which the technique can be taken to the field.

2.2 *Computation of formula*

For all practical purposes the discharge of the river water through the bucket has to be calculated first.

2.2a *Calculation of discharge through the bucket*: Discharge (D) is expressed as; $A \times c \times V$ (1)*, where A, c and V are, area of the bucket, a constant** and velocity of

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*Equation (1) has been adopted from Welch (1948) and presented here in a simplified form.

**The value of '*c*' is 0·8 if the river bottom is rough, loose, rocks, stones and coarse gravel and 0·9 if sandy or muddy. The former is true for the fluvial systems of Garhwal Himalaya.

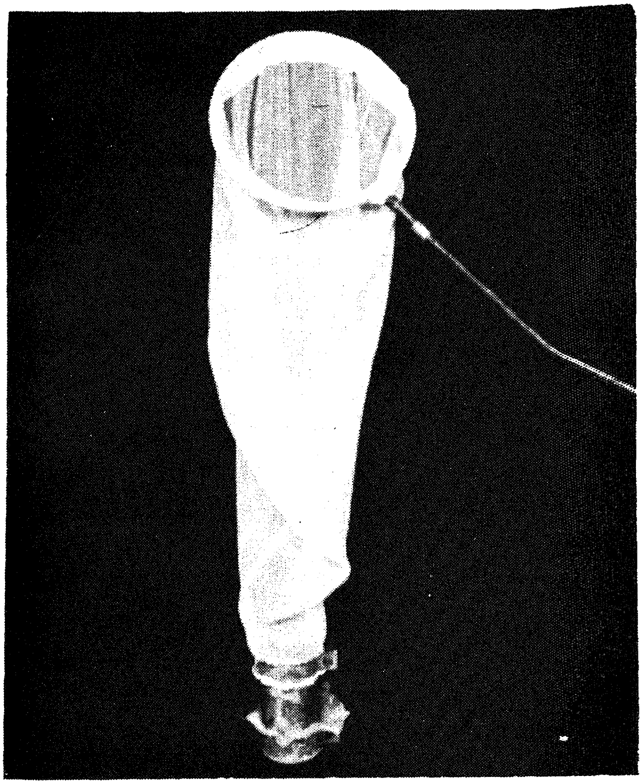

Figure 1. Conicol plankton net.

the river water, respectively. Since calculation of the area 'A' of the bucket is based on the formula πr^2, the radius of the bucket was measured (0·04 m in present case) and the equation (1) rewritten as;

$$\begin{aligned} D\,(\text{cumecs}) &= \pi r^2 \times c \times V \\ &= 3{\cdot}14(0{\cdot}04)^2 \times 0{\cdot}8 \times V \\ &= 0{\cdot}0040192\ V. \end{aligned} \tag{2}$$

2.2b *Estimation of time period*: This is the first major step which facilitates conversion of discharge 'D' into time period 't', the factor of prime significance in this method. This was achieved in the following manner;

$$D\,(\text{cumecs})^* = 0{\cdot}0040192\ V \tag{3}$$

or

$$(\text{litres/sec}) = 4V \tag{4}$$

$$t\,(\text{sec}) = x/4V \tag{5}$$

where 'x' is the desired volume of water to be filtered through the bucket (lit).

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*1 cubic meter = 1000 litres.

2.2c *Plankton sampling*: The process of plankton sampling shall be completed in 3 phases.

(i) *Measurement of velocity*: In torrential reaches, the mechanical current meters are not of much practical value. Shooting current and high turbulence have been observed to prevent regular rotation of the bucket wheel. Hence a float method (Welch 1948) was used to measure the velocity of the section of the river to be sampled.
(ii) *Calculation of* 't': The velocity 'V' once recorded simplifies the procedure. Its value when fitted into the formula $t = x/4V$ easily computes the time 't' in seconds (the value of 'x' being known)*.
(iii) *Sample collection*: The bucket possessing a desired mesh bolting silk cloth at its caudal end is then extended into the river and dipped for 't' seconds so that the 'x' volume may get filtered. The sample thus collected may be processed further for qualitative and quantitative analysis.

2.3 *Validity of the method*

In order to establish the validity of discharge-time method 4 sampling stations at a distance of 25 m each were selected along the left bank of Alaknanda. At all the 4 sites 50 litres of river water was filtered firstly by collecting with a bucket of known volume and secondly by discharge-time method. The plankton counts were made by the help of Sedgewick-Rafter Cell and enumerated by the formula:

$$n\ (\text{units/litre}) = \frac{(a \times 1000)c}{l}\ \text{(Welch 1948).}$$

The difference in the counts was tested by 't' test and found to be insignificant (table 1), which may be attributed to errors while making plankton counts.

2.4 *Qualitative and quantitative analysis*

In the present study the diatoms constituted 83·05% of the total plankton. They were represented mainly by *Navicula*, *Nitzschia*, *Cymbella*, *Diatoma* and *Synedra*. The green algae which accounted for 13·61% exhibited a very narrow spectrum of species (*Cladophora*, *Spirogyra* and *Ulothrix*). The desmids accounted for 3·33% of the total population.

2.5 *Plankton sampling device for torrents*

The need for modifying the device has already been emphasised. In shooting current the conical plankton net with large surface area provides resistance and thus cannot be held stationary for a long time. In order to offer minimum resistance, as already suggested under discharge–time method, only the detachable bucket of the plankton net should be used.

As plankton samples are usually collected from the river bank (for reasons already

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*The desired volume of water 'x' to be filtered shall depend on plankton density in the water body.

Table 1. Characteristic features of the sampling spot and statistics of plankton sampling.

	Sampling sites			
	1	2	3	4
River bed	Rough	Rough	Rough	Rough
Composition	Stones	Gravel, Pebbles, Stones	Gravel, Pebbles, Stones	Stones, Gravel
Turbulence	High	Slight	Nil	Normal
Velocity (V–m/sec)	0·55	0·40	0·45	0·52
Volume of sample to be filtered (x–litres)	50	50	50	50
Time ($t\,(\text{sec}) = x/4V$)	22·73	31·25	27·78	24·04
Plankton (units/litre)				
Manually	1140	2500	1820	1365
Discharge–Time Method	1020	2540	1780	1405
't' test Observed		0·22*		
't' test Table 5%		2·36		

*Insignificant

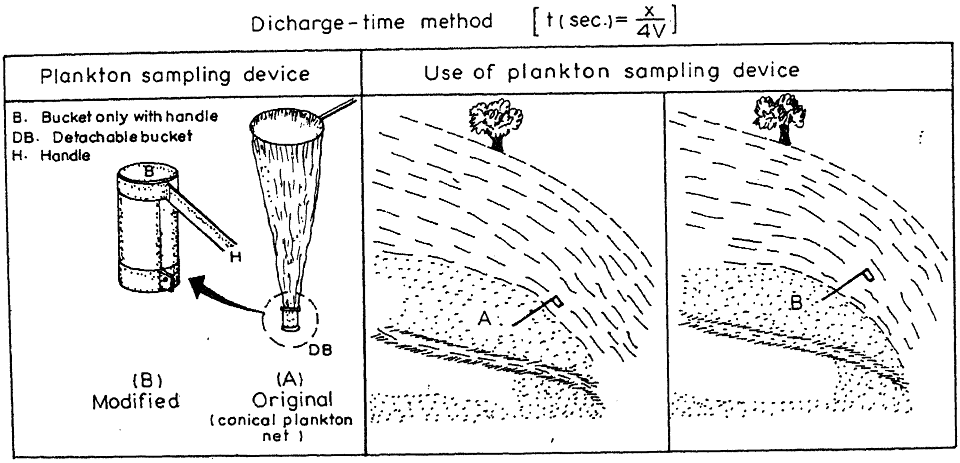
Dicharge - time method $\left[t\,(\text{sec.}) = \frac{x}{4V} \right]$
Plankton sampling device
Use of plankton sampling device
B. Bucket only with handle
DB. Detachable bucket
H. Handle
B
H
DB
(B) Modified
(A) Original (conical plankton net)
A
B

Figure 2. Discharge–Time Method.

mentioned in the text), in order to have a better sample, the bucket must be provided with a 1–1·5 meter long handle (wooden/metallic), which can fit into the bolt provided in the collar of bucket (figure 2).

In order to know the discharging velocity through the bolting silk, a meter or an electronic gadget shall have to be fitted at its caudal end, which shall record the discharge and enhance its efficiency.

The above account is, thus, a preliminary attempt to simplify the sampling process and enhance its efficiency in the torrential reaches.

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Factors affecting pollination activity of *Megachile lanata* lepel

D P ABROL* and R P KAPIL**
Laboratory of Animal Behaviour and Simulated Ecology, Department of Zoology, Haryana Agricultural University, Hisar 125 004, India
*Present address: Division of Entomology, S K University of Agricultural Science & Technology, Shalimar Campus, Srinagar (J and K) 191 121, India
**Present address: Director, Indian Lac Research Institute (ICAR), Namkum, Ranchi 834 010, India

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Abstract. Foraging activity of *Megachile lanata* on *Crotalaria juncea* L. flowers occurred only when minimum threshold of environmental factors was surpassed, while the cessation of activities was governed mainly by the fast decline in values of light intensity and solar radiation. Between the initiation and cessation of field activity, foraging population showed a positive correlation with air temperature, light intensity, solar radiation and nectar-sugar concentration fluctuations, but was negatively correlated with relative humidity, soil temperature and wind velocity. The path coefficient analysis revealed that direct effect of solar radiation and light intensity on foraging population were much pronounced and positive, while the effects of other factors were of very low magnitude, negative or negligible. However, their interrelationships caused accentuation of the overall effects of air temperature, relative humidity and nectar-sugar concentration. The resultant correlation with bee activity were strengthened through their favourable or unfavourable interaction with other factors.

Keywords. Environmental factors; *Megachile lanata*; *Crotalaria juncea*; pollination; path analysis.

1. Introduction

The pollinating effectiveness of bees depends upon their foraging population in the field and their behaviour on a crop. Since the field population as well as individual's foraging behaviour result from their innate and unavoidable responses to various environmental stimuli, ecological investigations on qualitative and quantitative aspects of their foraging on economic crops need to be thoroughly understood. This will help in assessment of the resultant interrelationships for their effective management and utilisation as prospective pollinators. Though pollination effectiveness of various bee pollinators has extensively been studied in the past, the studies are mainly based on a limited set of environmental conditions. For instance, Cirudarescu (1971) found that the number of insect visitors on lucerne was directly related to temperature and inversely to relative humidity, but the latter authors found it unaffected by relative humidity and vapour pressure. The flower visiting speed of honey bees increased with temperature (Benedek 1972; Burill and Dietz 1981). Wrona (1973) stated that pollination efficiency of honeybees increased with temperature and decreased with wind speed. Szabo and Smith (1972) found that foraging activity was positively correlated with a suitable combination of light intensity and temperature combined but not with the light intensity independent of temperature. Nunez (1977) reported that the morning activity was related to nectar flow and in the evening it was correlated with photoperiod. These reports indicate no fair general pattern of effects of different environmental factors influencing the pollination activity of bees. The probable reason seems in omission of such contributing factors actually influencing the pollination activity and in random selection of a few convenient ones having either no interaction or working in diverse ways, generating thus inconclusive results. Since environment is a complex of interrelated factors which influence the bee activity, and also plant physiology, a study of a complex phenomenon like pollination shall remain incomplete until a sizeable matrix of factors are taken on ensanable. These were the reasons for the present investigations which include air temperature, relative humidity, light intensity, solar radiation, nectar-sugar concentration, soil temperature and wind velocity as factors probably influencing bee-flower relationships.

2. Materials and methods

This study was conducted during September 1984 in sub-tropical Hissar located in the North Western plains of the country between 28° 59′–29° 46′ N (latitude) and 75° 11′–76° 18 E (longitude) at 215·2 m (altitude) on the Plant Breeding Farm of Haryana Agricultural University, Hissar where *Crotalaria juncea* is grown regularly for seed production. Hourly observations starting from 0800–1800 h on population/m^2 of *Megachile lanata* were recorded on *C. juncea* following Sihag (1982). Concurrent with the forager counts hourly measurements of environmental factors were made in the experimental field. Atmospheric temperature (T) and relative humidity (RH) were measured with a ‘dry and wet’ bulb thermometer. Soil temperature (ST) was recorded with a soil thermometer at a depth of 25 cm. Light intensity (LI) was recorded with a Luxmeter, model Luxomet–300 manufactured by M/s Research Instrumentation, Naraina Industrial Area, New Delhi. Similarly solar radiation (SR) was recorded by solarimeter, model SM 201, manufactured by M/s Central Electronics Ltd., Sahibabad, UP. Wind velocity (WV) was recorded with a hand Anemometer manufactured by M/s Research Instrumentation, Naraina Industrial Area, New Delhi held vertically at a height of about 1 meter above the crop surface. Total dissolved solids in the nectars (NSC) were estimated with the pocket refractometers (model 1093, Range, 0–50%; model 312, Range, 40–85%) manufactured by M/s Toshniwal Brothers Pvt. Ltd., New Delhi. The recorded data were analysed on TDC-316 computer for simple correlation and path coefficient analysis following Dewey and Lu (1959).

3. Results

3.1 *Commencement and cessation of foraging activity*

The data in table 1 show the temperature, relative humidity, light intensity, solar radiation, soil temperature and wind velocity in relation to the commencement and cessation of field activity by *M. lanata* on *C. juncea* flowers during September 1984. The time at which the commencement of field activity occurred varied from one day to another, which was perhaps dependent upon the attainment of minimum threshold conditions required for the initiation of field activity.

Table 1. Temperature, relative humidity, light intensity, solar radiation, soil temperature and wind velocity in relation to the commencement and cessation of field activity of *M. lanata* on *C. juncea*.

Date of observations (Sept. 1984)	Commencement							Cessation						
	Time (h)	T (°C)	RH (%)	LI (lx)	SR (mw/cm^2)	ST (°C)	WV (kmph)	Time (h)	T (°C)	RH (%)	LI (lx)	SR (mw/cm^2)	ST (°C)	WV (kmph)
11	0855	22·5	75·00	2700	41·00	22·00	3·70	1820	26·00	78·00	2000	35·00	35·00	3·0
14	0810	27·5	80·00	2100	30·00	25·00	3·00	1825	28·50	74·00	1600	20·00	20·00	3·5
19	0750	28·0	82·00	2600	25·00	29·00	3·50	1810	30·00	65·00	1800	22·00	30·5	3·5

Table 2. Estimates of correlation coefficients exhibiting interrelationships of different environmental factors influencing pollination activity of *M. lanata*.

Factors	Relative humidity	Light intensity	Solar radiation	Nectar-sugar concentration	Soil temperature	Wind velocity	Bee activity
Air temperature	−0·359*	0·690**	0·0670	0·547**	−0·176	−0·264	0·650**
Relative humidity		−0·0661	0·833**	−0·871**	0·977**	0·987**	−0·357*
Light intensity			0·462**	0·364*	0·0632	0·0040	0·826**
Solar radiation				−0·542**	0·880**	0·865**	0·1548
Nectar-sugar concentration					−0·819**	−0·810**	0·584**
Soil temperature						0·984**	−0·260
Wind velocity							−0·286

$^*P \leqslant 0{\cdot}05$, $n-2=31$ $\quad$ $^{**}P \leqslant 0{\cdot}01$, $n-2=31$.

Variation in commencement of the field activity, however, could not be explained by any one factor. A complex combination of various factors was perhaps responsible for initiation of field activity. The studies further revealed that, in general a temperature of 22·5°C, 75% relative humidity, 2100 lx light intensity, 25 mw/cm^2 solar radiation, 22°C soil temperature and 3·0 kmph wind velocity appeared to be the minimum threshold conditions for initiation of field activity. The cessation of activity was, however, governed mainly by the fast decline in values of light intensity and solar radiation, which were appreciably low than that required for commencement of field activities. It was found that cessation of activities occurred even before the air temperature dropped to the values associated with the commencement of field activity (22·5°C).

The critical examination of the data further revealed the occurrence of factor compensatory mechanism for initiation of bee activity within the foraging ranges of the bees, where low values of one factor were compensated by the higher values of others. For instance, on 11 September 1984, foraging flight occurred when air temperature was 22·5°C light intensity 2700 lx and solar radiation 41 mw/cm^2, while on 14 September 1984, it started at a higher temperature (27·5°C) when values of other factors like light intensity (2100 lx) and solar radiation (30 mw/cm^2) were comparatively lower than those required for the initiation of field activity on 11 September 1984. Similar situation was observed on 19 September 1984, when the foraging flight commenced at 28°C, but the values of light intensity (2600 lx) and solar radiation (25 mw/cm^2) were lower than those associated with initiation of field activity on 11 September 1984. Evidently, the observations on 11 September 1984 indicate that low values of air temperature were compensated by higher values of light intensity and solar radiation while on 14 and 19 September, the reverse was true. Thus, existence of factor compensating mechanisms governing initiation of bee activity is a clear possibility.

3.2 *Diurnal trends in bee activity in relation to various environmental factors*

Hourly observations on bee counts showed that their abundance followed air temperature, light intensity, solar radiation, soil temperature and nectar-sugar concentration fluctuations but was inversely related to relative humidity and wind velocity (figure 1). Maximum foraging population of *M. lanata* was observed between 1200–1400 h when the air temperature ranged between 29·5–38°C, relative humidity between 45·0–67%, light intensity between 4700–7400 lx, solar radiation between 58·00–94·00 mw/cm^2, nectar-sugar concentration fluctuated between 46·30–54·2%, soil temperature between 25·00–36·00°C and wind velocity between 3·2–5–5 kmph. The conditions optimum for maximum bee activity lie between these values on all the days of observation.

3.3 *Relationship of foraging population of M. lanata with environmental factors*

Correlation coefficients between bee activity and 7 environmental factors were calculated. The data in table 2 showed the extent to which these factors were associated with number of *M. lanata* foraging on *C. juncea*. The results highlighted that within the limits of environmental conditions prevailing during the experimental

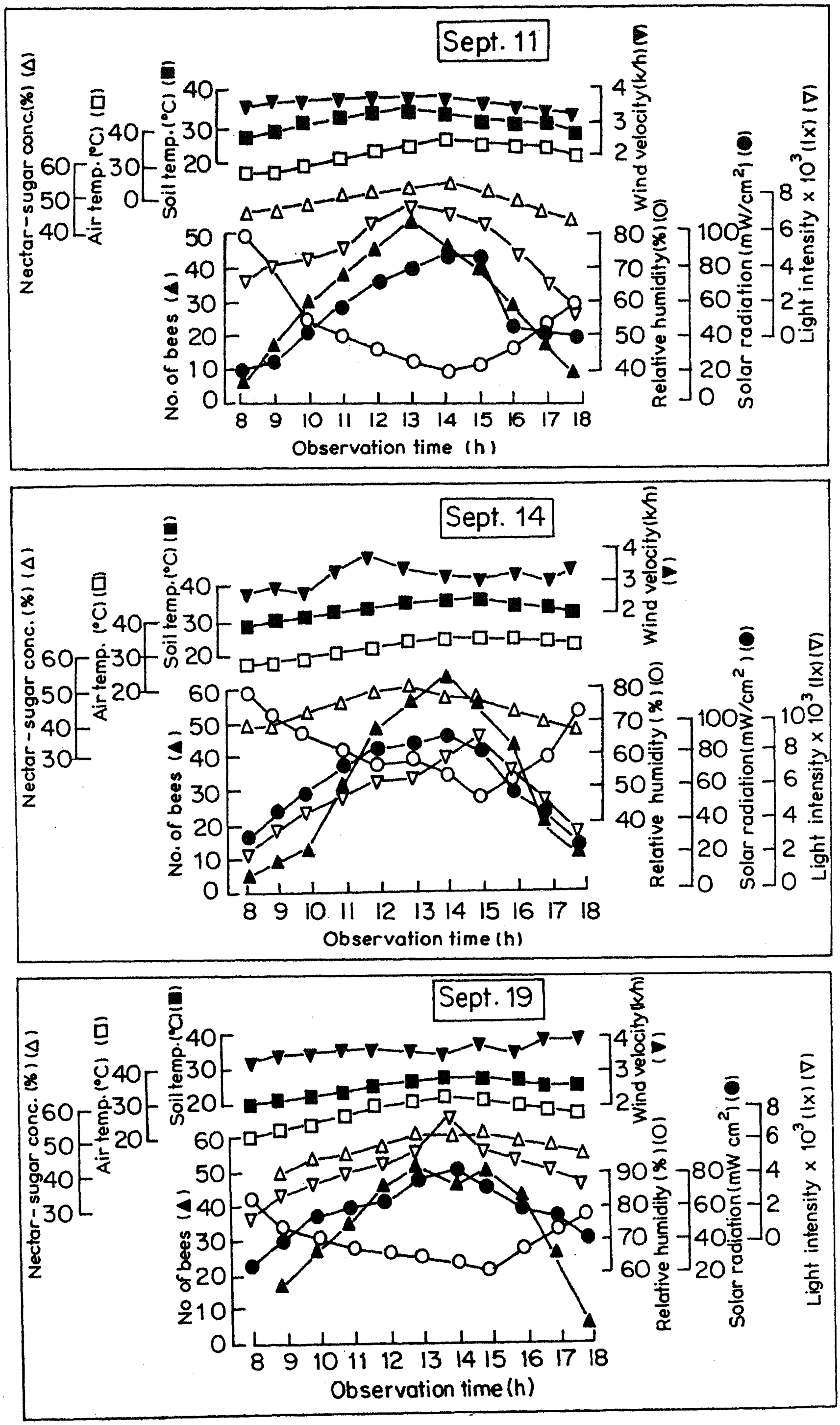

Figure 1. Diurnal trends in activity of *M. lanata* on *C. juncea* in relation to air temperature, relative humidity, light intensity, solar radiation, soil temperature, nectar-sugar concentration and wind velocity.

period, foraging population of *M. lanata* was significantly affected by higher values of air temperature, light intensity and nectar-sugar concentration fluctuations occurring together with lower values of relative humidity. The diurnal variations in soil temperature, solar radiation and wind velocity had a negligible effect on bee activity, and were not found to be associated with changes in the field population of *M. lanata.*

The environmental factors themselves are found to interact in several complex ways which is evident from their interrelationships. For instance, nectar-sugar concentration draw high positive correlation with temperature, solar radiation and nectar-sugar concentration while solar radiation make significantly positive correlation with relative humidity, soil temperature and wind velocity, but negative with nectar-sugar concentration. Soil temperature has a high positive correlation with relative humidity, solar radiation and wind velocity and significantly negative with nectar-sugar concentration. Likewise, wind velocity is highly and positively correlated with relative humidity, solar radiation and soil temperature but negative with nectar-sugar concentration.

Due to such complex web of interactions, the relationship between bee activity and environmental factors may not be direct as some of them are causal act directly whereas others may be influencing the activity indirectly, through the causal factors which further demanded investigation. For this purpose, correlations were further analysed by path coefficient technique. This technique involves the partitioning of the apparent association of different factors into direct effects (unidirectional pathways, *P*) and indirect effects (alternate pathways through factor interaction, pathways, $P \times$ correlation coefficient, *r*) and thus facilitates precise determination of relative importance of each factor. Bee activity was considered as the resultant variable and air temperature, relative humidity, soil temperature, wind velocity, light intensity, solar radiation and nectar-sugar concentration as the causal variables. Path coefficients were obtained by solution of a simultaneous equations through the method of least squares as given by Dewey and Lu (1959). The path coefficient analysis of interrelationships of temperature, relative humidity, light intensity, solar radiation, nectar-sugar concentration, soil temperature and wind velocity is shown by a schematic model (figure 2). The double-arrow lines indicate the mutual association as measured by correlation coefficients and the single-arrow lines represent direct effects in one direction as measured by path coefficients. The residual factors unaccounted for and considered independent of other variables are represented as px 8. The estimates of direct effect path coefficients and indirect effect path coefficients are presented in table 3.

3.4 *Bee activity versus air temperature*

The direct effect of air temperature on bee visits was positive (0·07946) but of very low magnitude and more or less negligible. It affected positively the bee activity, indirectly through relative humidity (0·4216), light intensity (0·3113), solar radiation (0·06004), and soil temperature (0·2164). But a strong negative influence of air temperature on bee visits was registered indirectly through nectar-sugar concentration (0·1463) and wind velocity (0·2921). Its negative interactions with nectar-sugar concentration and wind velocity tend to reduce its overall influence on bee activity,

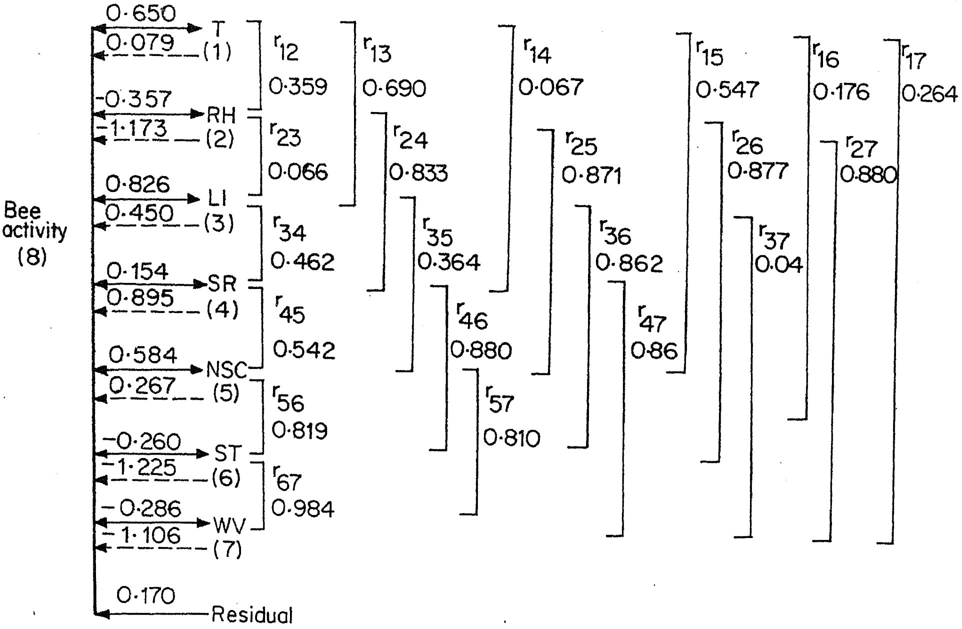

Figure 2. Schematic model exhibiting interrelationships of different environmental factors with bee activity.

but its overall positive association with bees ($r = 0{\cdot}650$) was largely a reflection of its favourable interactions with other factors.

3.5 *Bee activity versus relative humidity*

The relative humidity had a strong negative direct effect (1·1737) on the bee activity. However, its positive effect on the bee activity was indirect via solar radiation (0·7458), nectar-sugar concentration (0·2329), and wind velocity (1·092). The positive interactions of relative humidity with these factors reduce its overall negative effect. However, it affected negatively the bee activity indirectly via soil temperature (−1·1972) and air temperature (−0·02854). Its overall negative association with bees ($r = -0{\cdot}357$) was strengthened through its negative interactions with air and soil temperatures.

3.6 *Bee activity versus light intensity*

Simple correlation between the bee activity and light intensity was very high and positive (0·826). It was made up largely of the positive direct effect of light intensity (0·4509) and positive indirect effect via solar radiation (0·4139) on bee activity. Light intensity positively affected the bee activity indirectly via air temperature (0·0548), relative humidity (0·0776) and wind velocity (0·00440) by a negligible magnitude. Its negative indirect effects via nectar-sugar concentration (−0·0975) and soil temperature (0·0773) on the bee activity were relatively unimportant being negligible.

Table 3. Path coefficient analysis of bee activity vs air temperature, relative humidity, light intensity, solar radiation and nectar-sugar concentration, soil temperature and wind velocity.

Path ways of association	Direct effect path coefficient (*P*)	Indirect effect path coefficient (*P* × *r*)	Correlation coefficient (*r*)
1. Bee activity vs air temperature			0·650
Direct effect	0·0794		
Indirect effect via RH		0·4216	
” ” LI		0·3113	
” ” SR		0·0600	
” ” NSC		−0·1463	
” ” ST		0·2164	
” ” WV		−0·2921	
Total			0·650
2. Bee activity vs relative humidity			−0·357
Direct effect	−1·1737		
Indirect effect via T		−0·0285	
” ” LI		−0·0298	
” ” SR		0·7458	
” ” NSC		0·2329	
” ” ST		−1·1972	
” ” WV		1·0925	
Total			−0·357
3. Bee activity vs light intensity			0·826
Direct effect	0·4509		
Indirect effect via T		0·0548	
” ” RH		0·0776	
” ” SR		0·4137	
” ” NSC		−0·0975	
” ” ST		−0·0773	
” ” WV		0·0044	
Total			0·826
4. Bee activity vs solar radiation			0·1548
Direct effect	0·8953		
Indirect effect via T		0·0053	
” ” RH		−0·9778	
” ” LI		0·2083	
” ” NSC		0·1450	
” ” ST		−0·0790	
” ” WV		0·9576	
Total			0·1548
5. Bee activity vs nectar-sugar concentration			0·584
Direct effect	−0·2674		
Indirect effect via T		0·0434	
” ” RH		1·0223	
” ” LI		0·1644	
” ” SR		−0·4854	
” ” ST		1·004	
” ” WV		−0·8971	
Total			0·584

Table 3. (*Contd.*)

Path ways of association	Direct effect path coefficient (*P*)	Indirect effect path coefficient (*P* × *r*)	Correlation coefficeient (*r*)
6. Bee activity vs soil temperature			−0·260
Direct effect	−1·2250		
Indirect effect via T		−0·0140	
” ” RH		−1·1470	
” ” LI		0·0284	
” ” SR		0·7886	
” ” NSC		0·2196	
” ” WV		1·0890	
Total			−0·260
7. Bee activity vs wind velocity			−0·286
Direct effect	−1·1062		
Indirect effect via T		−0·2098	
” ” RH		−1·1592	
” ” LI		0·1017	
” ” SR		1·2173	
” ” NSC		1·8860	
” ” ST		−1·2048	
Total			−0·286
8. Residual			0·170

3.7 *Bee activity versus solar radiation*

The direct effect of solar radiation on the bee activity was much pronounced and positive (0·8953). Solar radiation positively affected the bee activity indirectly through air temperature (0·00532), light intensity (0·2083), nectar-sugar concentration (0·1450) and wind velocity (−0·9576). It was however negative indirect effect on the bee activity through relative humidity (0·9778) and soil temperature (−1·079). The negative interaction of these factors tended to reduce the overall influence of solar radiation on the bee activity. The total correlation between bee activity and solar radiation ($r=0·1548$) was largely due to its negative interaction with relative humidity and nectar-sugar concentration.

3.8 *Bee activity versus nectar-sugar concentration*

Surprisingly, the direct effect of nectar-sugar concentration on the bee activity was negative (−0·2674). Nectar-sugar concentration negatively affected the bee activity indirectly through solar radiation (−0·4854) and wind velocity (−0·8971). But these negative effects were diluted by its positive indirect effects on the bee activity through air temperature (0·04347), relative humidity (1·0223), light intensity (0·1644) and soil temperature (1·004). The overall significant yet positive contribution of nectar-sugar concentration to bees activity ($r=0·584$) was supported by its favourable interactions with air temperature, relative humidity, light intensity and soil temperature.

3.9 *Bee activity versus soil temperature*

The direct effect of soil temperature on the bee activity was strongly negative (−1·2250). Its indirect effects on the bee activity was positive via light intensity (0·02848), solar radiation (0·7886), nectar-sugar concentration (0·2192) and wind velocity (1·089). Its indirect negative influence on the bee activity was reckoned through air temperature (−0·0140) and relative humidity (−1·1470). The negative interaction of soil temperature with solar radiation was largely responsible for its overall negative association with the bees' activity ($r=0{\cdot}2607$).

3.10 *Bee activity versus wind velocity*

The correlation coefficient between wind velocity and the bee activity was moderately low and negative (−0·286). But its indirect effects on the bee activity was strongly negative (−1·1062), which it did through air temperature (−0·02098) relative humidity (−1·1592) and soil temperature (−1·2048) and positively via light intensity (0·1017), solar radiation (1·2173) and nectar-sugar concentration (1·8860). The net effect of these opposing influences was that 3 negative ones counter balance against the 3 positive ones, making overall correlation between wind velocity and bee activity negative ($r=-0{\cdot}286$). An overall examination of the correlation components revealed that solar radiation exerted the greatest positive influence on bee activity followed by light intensity, while soil temperature, wind velocity, nectar-sugar concentration and relative humidity negatively affected the bee activity directly. The direct effect of air temperature was almost negligible; however, it strongly affected the bee activity indirectly.

4. Discussion

The reproductive success and perpetuation of cross-pollinated plant species depend heavily on the abundance of associated pollinators and their pollinating efficiency which is under short duration turn-key control of the physical environment. Each bee species is guided by the specific ecological threshold for normal foraging activity whose maintenance differ inter and intra specifically according to their adaptability (Abrol 1985). *M. lanata* responds to increasing temperature in the morning until it touches 22·5°C which initiated the field activity. The ambient temperature was found to be the predominant factor governing morning flight of *M. rotundata* (Osgood 1974; Lerer *et al* 1982). But the latter authors observed that once the flight is initiated and temperature is maintained, it is solar irradiance that controls the pollination activity. According to Szabo and Smith (1972) it occurs due to combined effect of temperature and light intensity but in no case they work independent of each other. Karmo (1961) could place premium on temperature for initiation of foraging activity of *Apis mellifera* but not for its cessation. Observations by Cirusdarescu (1972) indicate that number of insect visits on crop was directly related to temperature and inversely to relative humidity. Wrona (1973) found pollinating activity of honey bees increased with temperature and decreased with wind speed. An increase in wind velocity has been reported to cause almost directly proportional reductions in the number of *A. mellifera* visiting apple bloom (Brittain 1935) and wind velocities above

17 kmph have been observed to greatly hamper their pollen gathering activities during Spring (Rashad 1957). The present investigations differ from these reports possibly because during the present studies wind invariably remained below 17 kmph. When acting in combination, the joint influence of other factors may have been far more strong and favourable to the foraging bees, and they may have masked any negative effect of wind on diurnal basis. However, changes in wind velocity when other factors are nearly constant can be expected to be indirectly proportional to population of foraging bees (Brittain 1935; Rashad 1957). Corbet (1978) attributed the changes in the spectrum of flowers visitors to fluctuations in nectar-sugar concentration.

The cessation of activity by *M. lanata* occurred when light intensity and solar radiation dropped for critical values although temperature was yet adequate to keep going the activity.

Once the minimum threshold conditions for initiation of activity were exceeded foraging population followed air temperature, light intensity, solar radiation, soil temperature and nectar-sugar concentration fluctuations but was inversely related to relative humidity and wind velocity. In general, maximum population was observed between 1200–1400 h, when the air temperature ranged between 29·5–38°C, light intensity between 4700–7400 lx, solar radiation between 58·0–94·0 mw/cm^2, relative humidity 45–67%, nectar-sugar concentration between 46·30–54·20, soil temperature between 25·0–36 and wind velocity between 3·2–5·5 kmph and the effects of other factor was negligible. The solar radiation could hardly be of any value independently as the bee activity never occur at 16–17°C. And it exerts influence only after 22·5°C as the minimum temperature threshold struck. Obviously, major contribution to the extent of 83% in causation of foraging activity is explained by environmental factors studies, remaining 17% seems to be due to atmospheric pressure, caloric award and also unknown factors. It is thus evident that air temperature provides a minimum threshold for initiation of flights, hence pollination activity of *M. lanata* and once flight is initiated and temperature level is kept up, solar radiation and light intensity takes over. The conditions optimum for maximum bee activity may however vary depending upon geographical regions, time of the year, crops or species of bees. For example, Cherian *et al* (1947) found 20·5 as the range of temperature suitable for the activity of *Apis cerana indica* on cotton crop at Coimbatore (South India), Kapil and Brar (1971) recorded peak activity of *A. florea* on *B. compestris* var *toria* when the temperature ranged between 21–25°C and relative humidity 50–57 at Ludhiana (North India) during November. Dhaliwal and Bhalla (1983) reckoned maximum population of *A.c. indica* on cauliflower when the air temperature ranged between 25·1–27·6°C, light intensity between 2000–3233 lx and relative humidity 26–29·4% during April at Solan (North India). Factor compensating mechanisms were also observed operating the commencement of pollination activity of *M. lanata* in which lower values of temperature were compensated jointly by higher values of light intensity and solar radiation and vice versa. A similar phenomenon has been reported by Szabo and Smith (1972) who found that *M. rotundata* foraged at 1075 lx when the temperature was 25°C but needed 6450 lx at 17°C.

In contrast, the cessation was killed by light intensity (Osgood 1974) or by evening radiations dropping to 0·3 langleys normally or even by intervening clouds (Gerber and Klostermeyer 1972).

The direct and indirect influences of various environmental factors as measured by path coefficient analysis indicated that light intensity and solar radiation exerted the greatest positive influence directly. This may in part be due to their direct influence on the physiological and metabolic processes of the plants which are known to yield higher amounts of nectar and pollen at relatively higher values of these factors, while the other factors, viz soil temperature, wind velocity, relative humidity and nectar-sugar concentration negatively affected the bee activity. The direct effect of air temperature was nearly negligible, however, it strongly affected the bee activity indirectly through other factors.

It is thus evident that path coefficient analysis yielded an entirely different picture of the effects than by simple scatter diagrams or simple correlations used by earlier workers (Benedek and Prener 1972; Kapil and Brar 1971; Nunez 1977; Szabo and Smith 1972; Corbet 1978). For instance, simple correlation between bee activity and air temperature was highly significant and positive ($r = 0{\cdot}650$) which means that with increase in temperature bee visits would increase provided other variables are maintained constant. But path coefficient analysis, however revealed little effect of air temperature on bee activity as its direct effect was of very low magnitude (0·0794). Its overall significant positive association with bees ($r = 0{\cdot}650$) was strengthened through its favourable interactions with other factors. Similarly, simple correlation between the bee activity and solar radiation ($r = 0{\cdot}1548$) is having little influence on the bee activity. The path coefficient enhanced its influence on foraging population as pronounced and positive (0·8953). The accentuation of its overall effect was caused by its negative interactions with other factors. Likewise, widely held contention that nectar-sugar concentration directly influences the bee activity (Corbet 1978) is also belied by this analysis.

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Proceedings (Animal Sciences)

Volume 95, 1986

SUBJECT INDEX

Abiotic factor
  A study on the effects of certain abiotic factors on the activity of *Adoretus epipleuralis* Arrow (Coleoptera: Scarabaeidae) 97
Absorption efficiency
  Prediction of absorption efficiency from food nitrogen in amphibians 387
Abundance
  Distribution and abundance of prawns in the freshwater habitats of Bangalore, South India 77
Acanthamoeba culbertsoni
  Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures 469
Acanthamoeba rhysodes
  Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures 469
Acid phosphatase
  Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina* 411
Adaptedness
  Adaptedness of five closely related strains of *Drosophila* to media containing molasses or fructose 557
Adipocyte histomorphology
  Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats 45
Adipocyte number
  Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats 45
Adipocyte size
  Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats 45
Adoretus lasiopygus
  Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera) 205
Adrenal
  Testicular damage in rats fed on irradiated diets 705
Adrenal medulla
  Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons 223
Adult nutrition
  Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) 677
Advantages of cephalic expansion
  Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions 509
Aedeagus
  Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records 487
Aeration
  Effect of artificial aeration on the growth and survival of Indian major carps 371
Agromyzidae
  Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records 487
Air-lift system
  Effect of artificial aeration on the growth and survival of Indian major carps 371
Alactomy
  Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes 613
Albino rats
  Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats 263
Alfalfa
  Time and energy budgets of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) 579
Ambient oxygen
  Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67
Ammonia quotient
  Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67
Anabolic steroid
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51
Analysis of covariance
  Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae) 187

Anisops bouvieri predation
  Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions 509
Antennectomy
  Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes 613
Aphid
  Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region 181
Apis florea F.
  A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Appetite stimulant
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51
Aquaculture and ranching
  Production and utilization of frogs: an ecological view 289
Ariidae
  Evolution and biogeography of the Indian genera of the family Ariidae 279
Assimilation efficiency
  Prediction of assimilation efficiency of lepidopterans 641
Auto-heterosynthesis
  Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca) 171

Bait preference
  Laboratory evaluation of bait base for the control of sand coloured rat, *Rattus gleadowi* 417
BASF LAB 153 959 I
  Effect of an insect growth regulator on the growth and development of the tobacco caterpillar, *Spodoptera litura* Fabricius 689
Benthic fauna
  Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325
Biochemical constituents
  Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos) 539
Biological activity of wormcasts
  Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts 341
Biology
  Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region 181
Biomass
  Composition and variations in the abundance of zooplankton in the Hooghly estuary, West Bengal, India 125
  Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325
Blood cells
  Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio* 617
Blood glucose
  Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation 723
Blood protein
  Changes in the Haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713
Body size
  Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247
Brachionus quadridentatus
  Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India) 365
Bright-, dim- and dark-adaptation
  Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) 59
Brodifacoum
  Effects of ecological factors on the performance of rodenticides in sugar cane 729

Carbohydrate metabolism
  In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure 525
Carrot seed extract
  Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats 263
Catch
  A study on the effects of certain abiotic factors on the activity of *Adoretus epipleuralis* Arrow (Coleoptera: Scarabaeidae) 97
Catla catla
  Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) 103
  Effect of artificial aeration on the growth and survival of Indian major carps 371
Catopsilia crocale
  Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) 303

Cavariella aegopodii
  Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region 181
Cercariae
  Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy 623
Channa punctatus
  Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch) 719
Channa striatus
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51
Characterization
  Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes 255
Chi-square test
  Some observations on the biology of periwinkle *Littorina scabra scabra* (Linne 1758) 269
Chorion
  Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) 237
  Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta: Heteroptera) 447
Chronic influence
  Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson) 515
Cirrhinus mrigala
  Effect of artificial aeration on the growth and survival of Indian major carps 371
Clam bed
  Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325
Clarias batrachus
  Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets 457
  Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes 255
Clibanarius olivaceus
  Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson) 515
Coconut rhinoceros beetle
  Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) 379
Co-culture
  Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures 469
Competition
  Competition studies in *Drosophila*: Relative fitness and adaptedness of six closely related species 199
Composition
  Composition and variations in the abundance of zooplankton in the Hooghly estuary, West Bengal, India 125
Composition of wormcasts
  Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts 341
Conservation
  Production and utilization of frogs: an ecological view 289
Continuous flow
  Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch) 719
Conversion efficiency
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51
Copper
  Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713
Crabs
  Distribution and zonation of shore crabs in the Pulicat lake 437
Critical developmental stage
  Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) 379
Crotalaria juncea
  Factors affecting pollination activity of *Megachile lanata* lepel 757
Cubic value
  On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) 423
Cue
  Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89
Cypriniformes
  Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes 255
Cyrtacanthacris ranacea
  Sensillar diversity, density and distribution during post-embryonic development of *Cyrtacanthacris ranacea* stoll and their role in feeding 117

Daphnia cephalata
  Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia*

cephalata King (Crustacea: Cladocera) under laboratory conditions 247

Daphnia cephalata King
  Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions 509

Daphnia similis
  Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) 163
  Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247

Daphnia similis Claus
  Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions 509

Defoliation dynamics
  The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths 7

Dendrite
  A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595

Developmental stages
  Seasonal effect on certain developmental stages of *Tricholyga bombycis* Beck. (Diptera: Tachinidae) 561

D1 diphenylamine
  Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton 147

Diflubenzuron
  Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton 147

Digestive organs
  Functional morphology, anatomy and histology of the digestive organs of the catfish *Plotosus canius* Hamilton 23

Digestive reducing substances
  Prediction of assimilation efficiency of lepidopterans 641

Discharge-time method
  Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device 751

Distillery and paper mill effluents
  Hematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters) 155

Distribution
  Distribution and abundance of prawns in the freshwater habitats of Bangalore, South India 77
  Distribution and zonation of shore crabs in the Pulicat lake 437

DNA
  Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton 147

Drosophila
  Competition studies in *Drosophila*: Relative fitness and adaptedness of six closely related species 199
  Adaptedness of five closely related strains of *Drosophila* to media containing molasses or fructose 557

Drosophila melanogaster
  Induction and genotypic verification of recombinants in males of *Drosophila melanogaster* 587

Dysdercus koenigii
  Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) 677

Earias vittella
  Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes 613

Earthworm casts
  Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts 341

Echtrichodiinae
  Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta- Heteroptera) 447

Economic importance
  Feeding ecology of the large grey babbler *Turdoides malcolmi* 429

Education and export sectors
  Production and utilization of frogs: an ecological view 289

Effect of MOC
  Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio* 617

Egg
  Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) 237

Egg output
  Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females

on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) 677

Egg production
  Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247

Egg production and body size
  Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) 163

Eggs
  Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta-Heteroptera) 447

Egg viability
  Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes 613
  Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) 677

Energy budget
  Time and energy budgets of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) 579

Energy metabolism
  Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403

Environmental factors
  Factors affecting pollination activity of *Megachile lanata* lepel 757

Environmental variability
  Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325

Enzymes level
  Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera) 205

Epinephrine-norepineephrine content
  Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons 223

Epithelial cell processes
  Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) 59

Esterase
  Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy 623

Ethylestrenol
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51

Faeces
  Occurrence of gastric absorption in the cichlid fish, *Sarotherodon mossambicus* 685

Faunal diversity
  Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325

Fecundity
  Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) 303

Feeding
  Sensillar diversity, density and distribution during post-embryonic development of *Cyrtacanthacris ranacea* Stoll and their role in feeding 117

Feeding ecology
  Feeding ecology of the large grey babbler *Turdoides malcolmi* 429

Fish
  Hematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters) 155

Fish haematology
  Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio* 617

Fitness
  Competition studies in *Drosophila*: Relative fitness and adaptedness of six closely related species 199

Flank gland
  Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667

Flight activity
  A study on the effects of certain abiotic factors on the activity of *Adoretus epipleuralis* Arrow (Coleoptera: Scarabaeidae) 97

Fluvial system
  Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device 751

Food nitrogen as index of Ae
  Prediction of absorption efficiency from food nitrogen in amphibians 387

Free-ranging rhesus monkey
  Ontogeny of play behavior in free-ranging rhesus macaques (*Macaca mulatta*) 605

Frog harvest
  Production and utilization of frogs: an ecological view 289

F-test
  Some observations on the biology of periwinkle *Littorina scabra scabra* (Linne 1758) 269

Gastric absorption
  Occurrence of gastric absorption in the cichlid fish, *Sarotherodon mossambicus* 685
Genotypic verification
  Induction and genotypic verification of recombinants in males of *Drosophila melanogaster* 587
Glutamate dehydrogenase
  Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure 533
Glycolysis
  In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure 525
Gonadectomy
  Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons 223
Gonad index
  Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast 1
Growth
  Growth and population dynamics of the Indian white prawn *Penaeus indicus* H M Edwards from Kakinada 629

Habitat area
  Production and utilization of frogs: an ecological view 289
Haematological indices
  Trypanosoma evansi infection and the influence of some hormones on haematological indices in albino rats 463
Haemolymph
  Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713
Harpactorinae
  Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) 237
Heliothis spp.
  Heliothis species and their natural enemies, with their potential for biological control 695
Hematology
  Hematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters) 155
Hepatopancreas
  Changes in the Haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713
Heteropneustes fossilis
  Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes 255
Hibernation
  Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403
Histogenesis
  Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton 147
Histolysis
  Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton 147
Hooghly estuary
  Composition and variations in the abundance of zooplankton in the Hooghly estuary, West Bengal, India 125
Hormone changes
  Trypanosoma evansi infection and the influence of some hormones on haematological indices in albino rats 463
Host range
  Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stal) 227
Host selection
  Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stal) 227
Host specificity
  Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stal) 227
Host visitation
  Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum City with a note on mite infestation 475
Hyblaea puera
  The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths 7
Hyperglycemic hormone
  Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation 723
Hypoxia
  Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67

Implantation
  Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats 263
Indian musk shrew
  Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667

Indotermitidae
  Wings and wing-microsculpturing in the termite family Indotermitidae (*Indotermes*, Isoptera) and their bearing on phylogeny 191
Induced recombination
  Induction and genotypic verification of recombinants in males of *Drosophila melanogaster* 587
Insect growth regulator
  Effect of an insect growth regulator on the growth and development of the tobacco caterpillar, *Spodoptera litura* Fabricius 689
Interrelationship of Indian genera
  Evolution and biogeography of the Indian genera of the family Aridae 279
Interscapular brown adipose tissue
  Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats 45
Intestine
  Occurrence of gastric absorption in the cichlid fish, *Sarotherodon mossambicus* 685
Irradiated diet
  Testicular damage in rats fed on irradiated diets 708

Labeo rohita
  Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) 103
  Effect of artificial aeration on the growth and survival of Indian major carps 371
Laboratory condition
  Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) 163
Laccase
  Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros* 739
Lactate dehydrogenase
  Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes 255
Large grey babbler
  Feeding ecology of the large grey babbler *Turdoides malcolmi* 429
Larval density
  Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India 549
Leaf mine
  Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records 487
Leaf water
  Prediction of assimilation efficiency of lepidopterans 641
Length-weight relationship
  Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae) 187
  On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) 423
Lepidopteran larvae
  Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) 303
Lepidopteran species
  Prediction of assimilation efficiency of lepidopterans 641
Life table
  Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) 303
Light microscopy
  A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Limiting factors
  The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths 7
Lipid oriented metabolism
  Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos) 539
Littorina scabra scabra
  Some observations on the biology of periwinkle *Littorina scabra scabra* (Linne 1758) 269
Logarithmic equation
  On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) 423
Longevity
  Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) 163
  Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247

Macrognathus aculeatus
  On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) 423
Male genitalia
  Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records 487
Males
  Induction and genotypic verification of recombinants in males of *Drosophila melanogaster* 587

Mansonia
Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city with a note on mite infestation 475
Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India 549
Mating trends
Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) 677
Mean days of moulting
Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson) 515
Megachile flavipes
Time and energy budget of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) 579
Megachile lanata
Factors affecting pollination activity of *Megachile lanata* lepel 757
Megachile nana
Time and energy budgets of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) 579
Meiotic chromosomes
Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera) 135
Meloidogyne incognita
Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita* 397
Metamorphic index
Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina* 411
Methods of estimation
Prediction of absorption efficiency from food nitrogen in amphibians 387
Midgut
Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera) 205
Migration
The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths 7
Mining rejects
Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325
Mitochondrial inclusions
Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667
Modified Banta's medium
Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247
Morpho-histology
Functional morphology, anatomy and histology of the digestive organs of the catfish *Plotosus canius* Hamilton 23
Morphometry
Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region 181
Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India) 365
Mortality rates
Growth and population dynamics of the Indian white prawn *Penaeus indicus* H M Edwards from Kakinada 629
Moult cycle
Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713

Natural enemies
Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region 181
Nemipterus japonicus
Visual capacity of marine teleost *Nemipterus japonicus* (Bloch) 567
Nervous system
Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy 623
Neuroendocrine regulation
Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation 723
Nitrogen excretion
Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid fish, *Oreochromis mossambicus* (Peters), with special reference to swimming speed 215
N. nigropictus
Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stal) 227
Normoxia
Influence of ambient oxygen and random swimming activity on metabolic rates and quotients

in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67
Nutrition
Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets 457
N. virescens
Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stal) 227

Ocypoda macrocera
Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast 1
Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713
Olfactory communication
Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89
Ontogeny
Ontogeny of play behaviour in free ranging rhesus macaques (*Macaca mulatta*) 605
Operculum
Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) 237
Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta-Heteroptera) 447
Oryctes rhinoceros
Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) 379
Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros* 739
Oviposition
Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes 613
Oxygen consumption
Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid fish, *Oreochromis mossambicus* (Peters), with special reference to swimming speed 215
Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403
Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos) 539

Parasitization
Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city with a note on mite infestation 475
Parasitoids
Heliothis species and their natural enemies, with their potential for biological control 695
Pathogens
Heliothis species and their natural enemies, with their potential for biological control 695
Penaeus indicus
In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure 525
Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure 533
Pest control
Production and utilization of frogs: an ecological view 289
Phenoloxidases
Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros* 739
Phenthoate
Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch) 719
Phosphamidon
In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure 525
Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure 533
Phylogeny
Evolution and biogeography of the Indian genera of the family Ariidae 279
Physico-chemical factors
Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India 549
Physico-chemical properties
The physico-chemical environment and the plankton of managed ponds in Haryana, India 353

Physopelta schlanbuschi
Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera) 135
Phyto plankton
The physico-chemical environment and the plankton of managed ponds in Haryana, India 353

Plankton
The physico-chemical environment and the plankton of managed ponds in Haryana, India 353

Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device 751

Plant growth promotors in wormcasts
Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts 341

Plant products
Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita* 397

Plotosus canius
Functional morphology, anatomy and histology of the digestive organs of the catfish *Plotosus canius* Hamilton 23

Pod set
Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants 745

Poison baiting
Effects of ecological factors on the performance of rodenticides in sugar cane 729

Pollen load
Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants 109
Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants 745

Pollination
Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants 109
Time and energy budgets of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) 579
Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants 745

Pollination path analysis
Factors affecting pollination activity of *Megachile lanata* lepel 757

Polymorphosis
Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India) 365

Pond
The physico-chemical environment and the plankton of managed ponds in Haryana, India 353

Pond water
Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247

Population dynamics
The teak defoliator, *Hyblaca puera*. Defoliation dynamics and evidences for short-range migration of moths 7
Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) 303

Prawns
Distribution and abundance of prawns in the freshwater habitats of Bangalore, South India 77

Precocious metamorphosis
Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) 379

Predators
Heliothis species and their natural enemies, with their potential for biological control 695

Progesterone
Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats 263

Protease
Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure 533

Protein requirement
Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) 103

Pulicat
Distribution and zonation of shore crabs in the Pulicat lake 437

Rainfall
Effects of ecological factors on the performance of rodenticides in sugar cane 729

Rana tigrina
Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina* 411

Rattus gleadowi
Laboratory evaluation of bait base for the control of sand coloured rat, *Rattus gleadowi* 417

Redia
Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy 623

Reduviidae
Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) 237
Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta-Heteroptera) 447

Regression coefficient
On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) 423

Reproduction
Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403

Reproductive cycle
Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast 1

Reptiles
Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403
Respiration
Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403
Respiratory enzymes
Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch) 719
Respiratory quotient
Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67
Retina
Visual capacity of marine teleost *Nemipterus japonicus* (Bloch) 567
Retinal epithelial pigment layer
Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) 59
Retinomotor responses
Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) 59
Rhinomugil corsula
Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67
Rice bran as food
Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) 163
Rodents
Effects of ecological factors on the performance of rodenticides in sugar cane 729
Rotifera
Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India) 365
RQ, AQ and NQ
Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid " fish, *Oreochromis mossambicus* (Peters), with special reference to swimming speed 215

Salinity
Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast 1
Sampling
Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device 751
Scarabaeidae
Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera) 205
Scent marking
Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89
Seasonal effect
Seasonal effect on certain developmental stages of *Tricholyga bombycis* Beck. (Diptera: Tachinidae) 561
Seasonal prevalence
Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city with a note on mite infestation 475
Sebaceous tubules
Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667
Self-play
Ontogeny of play behavior in free-ranging rhesus macaques (*Macaca mulatta*) 605
Sensilla
Sensillar diversity, density and distribution during post-embryonic development of *Cyrtacanthacris ranacea* Stoll and their role in feeding 117
Sensilla basiconica
A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Sensilla placodea
A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Sensilla trichodea
A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Shore
Distribution and zonation of shore crabs in the Pulicat lake 437
Social behaviour
Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89
Social play
Ontogeny of play behavior in free-ranging rhesus macaques (*Macaca mulatta*) 605
Solanaceae
Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants 109
Spodoptera litura
Effect of an insect growth regulator on the growth and development of the tobacco caterpillar, *Spodoptera litura* Fabricius 689
Starvation
Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) 379

Starvation stress
  Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos) 539
Static media
  Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch) 719
Steroid supplementation
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51
Stigmatic receptivity
  Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants 109
Substrate
  The physico-chemical environment and the plankton of managed ponds in Haryana, India 353
Sugar cane
  Effects of ecological factors on the performance of rodenticides in sugar cane 729
Sugars
  Adaptedness of five closely related strains of *Drosophila* to media containing molasses or fructose 557
Sulphated AMP
  Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca) 171
Suncus murinus viridescens
  Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667
Supra-oesophageal ganglion
  Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation 723
Survival rate
  Effect of zinc on zoeal development of the estuarine crab *Clibanarius olivaceus* (Henderson) 515
Swimming activity
  Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67

Teak defoliator
  The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths 7
Testes
  Testicular damage in rats fed on irradiated diets 705
Thrips
  Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants 109
  Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants 745
Thymolymphatic system
  Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons 223
T-maze
  Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89
Tormogen cell
  A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Torrential reaches
  Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device 751
Toxicity
  Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita* 397
Toxicology
  Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio* 617
Transmission electron microscopy
  A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Trematode
  Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy 623
Triatominae
  Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Echtrichodiinae (Insecta-Heteroptera) 447
Trichogen cell
  A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595
Tricholyga bombycis
  Seasonal effect on certain developmental stages of *Tricholyga bombycis* Beck. (Diptera: Tachinidae) 561
Tropical earthworms
  Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts 341
Tropical intertidal gastropod
  Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos) 539
Tropical marine invertebrates
  Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast 1
Trypanosoma evansi
  Trypanosoma evansi infection and the influence of some hormones on haematological indices in albino rats 463

't' test
  Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae) 187
Turdoides malcolmi
  Feeding ecology of the large grey babbler *Turdoides malcolmi* 429
Tyrosinase
  Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros* 739

Ultrastructure
  Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667
Urine marking
  Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89

Visual cell layer
  Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) 59
Visual cells
  Visual capacity of marine teleost *Nemipterus japonicus* (Bloch) 567
Vitamin C
  Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons 223

Wing microsculpturing
  Wings and wing microsculpturing in the termite family Indotermitidae (*Indotermes*, Isoptera), and their bearing on phylogeny 191
Wings
  Wings and wing microsculpturing in the termite family Indotermitidae (*Indotermes*, Isoptera), and their bearing on phylogeny 191

Xenentodon cancila
  Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae) 187
X-ray effects
  Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera) 135

Yield per recruit
  Growth and population dynamics of the Indian white prawn *Penaeus indicus* H M Edwards from Kakinada 629
Yolk granules
  Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca) 171

Zinc
  Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson) 515
Zinc phosphide
  Effects of ecological factors on the performance of rodenticides in sugar cane 729
Zooplankton
  Composition and variation in the abundance of zooplankton in the Hooghly estuary, West Bengal, India 125
  The physico-chemical environment and the plankton of managed ponds in Haryana, India 353

AUTHOR INDEX

Abrol D P
  Time and energy budgets of alfalfa pollinating bees *Megachile nana* Bingh and *Megachile flavipes* Spinola (Hymenoptera: Megachilidae) 579
  Factors affecting pollination activity of *Megachile lanata* lepel 757
Ahamed Nazeer R
  see Kaliwal B B 263
Ahmad Nafis
  see Parshad V R 729
Ajmalkhan S
  Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson) 515
Anand C S K
  see Kulkarni G K 723
Ananthakrishnan T N
  Sensillar diversity, density and distribution during post-embryonic development of *Cytacanthacris ranacea* Stoll and their role in feeding 117
Annadurai R S
  see Velayudhan R 109
  Pollination potential of thrips (Insecta: Thysanoptera) in some fabaceous plants 745
Ansari Z A
  see Parulekar A H 325
Aruchami M
  see Jeyaraj R 739
Arul V
  Effect of ethylestrenol on growth and food utilization in *Channa striatus* 51

Balakrishnan M
  Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth) 667
Balasubramonian N K
  see Chandrika B 187
Banerjee Samir
  see Homechaudhuri Sumit 617
Baskaran R
  Influence of light intensity on retinomotor responses in *Channa punctatus* (Bleeker) 59
Basurmanova O A
  see Balakrishnan M 667
Bhardwaj A C
  Digestive enzymes level in the midgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptera) 205
Bhatnagar V S
  see Pawar C S 695

Chakrabarti S
  see Ghosh D 181
Chandrika B
  Length-weight relationship of *Xenentodon cancila* (Ham.) (Teleostei: Belonidae) 187
Chand Rohtash
  A study on the effects of certain abiotic factors on the activity of *Adoretus epipleuralis* Arrow (Coleoptera: Scarabaeidae) 97
Chatterjee Shilla
  Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons 223
Chopra G
  see Parshad V R 729
Choubisa S L
  Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy 623
Choudhury A
  see Sarkar S K 125
Choudhury Amalesh
  see Ghosh Mrinal 469
Christopher M S M
  Population dynamics of a tropical lepidopteran *Catopsilia crocale* (Pieridae) 303

Daniel B Ayyachamy
  Seasonal prevalence and host visitation of *Mansonia* mosquitoes in Trivandrum city with a note on mite infestation 475
Davies R W
  see Singhal R N 353
Dechamma P A
  Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats 45
Dehadrai P V
  see Venkatesh B 457
Devi S Lalitha
  Growth and population dynamics of the Indian white prawn *Penaeus indicus* H M Edwards from Kakinada 629
Devi V Uma
  Starvation as a stress factor influencing the metabolism of tropical intertidal gastropod *Morula granulata* (Duclos) 539
Dey S K
  see Manna G K 135

Dhanze J R
see Jayaram K C 279

Ghosh Asok
see Chatterjee Shilla 223
Ghosh D
Holocycly, seasonal activity, morphometry and natural enemies of willow aphid, *Cavariella aegopodii* (Scopoli) (Homoptera: Aphididae) in the Indian region 181
Ghosh Mrinal
Growth of *Acanthamoeba culbertsoni* (Singh and Das 1970) and *Acanthamoeba rhysodes* (Singh 1952) (Protozoa: Gymnamoebia) in cultures 469
Gujar G T
Effect of an insect growth regulator on the growth and development of the tobacco caterpillar, *Spodoptera litura* Fabricius 689
Gupta B B P
Annual variation in the oxygen utilization dynamics of the garden lizard, *Calotes versicolor* 403
Gupta Mahavir
A quantitative study and ultrastructure of flagellar sensillae of *Apis florea* F. (Hymenoptera: Apidae) 595

Haniffa M A
Hematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters) 155
Haridass E T
Ultrastructure of the eggs of Reduviidae: II eggs of Harpactorinae (Insecta: Heteroptera) 237
Ultrastructure of the eggs of Reduviidae: III. Eggs of Triatominae and Ectrichodiinae (Insecta: Heteroptera) 447
Hasan S S
see Kushwaha A K S 705
Homechaudhuri Sumit
Effect of mahua oil cake on the blood cells and blood values of an airbreathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio* 617

Idris Mohd
Scent marking in the Indian gerbil, *Tatera indica* in response to conspecific odours 89
Laboratory evaluation of bait base for the control of sand coloured rat, *Rattus gleadowi* 417
Ingole B S
see Parulekar A H 325

Jadhav D R
see Pawar C S 695
Jain A P
see Idris Mohd 417
Jayaram K C
Evolution and biogeography of the Indian genera of the family Ariidae 279
Jeet Swarn
see Singhal R N 353
Jeyaraj R
Characteristics of phenoloxidases in larval cuticle of the coconut pest, *Oryctes rhinoceros* 739
Job S V
see Narayanan M 685
Joel D R
Distribution and zonation of shore crabs in the Pulicat lake 437
Jolly M S
see Kumar Pradip 561

Kaliwal B B
Implantation delay, nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats 263
Kalode M B
see Viswanathan Kasi 227
Kalyanaraman K
see Daniel B Ayyachamy 475
see Sasikumar P S 549
Kamath S
see Dechamma P A 45
Kameswari M
see Hemalatha M 463
Kapil R P
see Abrol D P 757
Kasinathan R
see Maruthamuthu S 269
Katre Shakuntala
see Raman K V Anantha 77
Kesary M
see Venkataraman K 163
Krishnamoorthy R V
Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts 341
Krishnamurthy N B
see Ranganath H A 199
Krishna S S
see Pathak P H 613
see Shahi K P 677
Krishnaswamy S
see Venkataraman K 163
see Venkataraman K 247
see Venkataraman K 509
Kulkarni G K
Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation 723
Kulkarni N
see Saksena D N 365
Kumar Pradip
Seasonal effect on certain developmental stages of *Tricholyga bombycis* Beck. (Diptera: Tachinidae) 561

Kumar Suresh N
see Ananthakrishnan T N 117
Kushwaha A K S
Testicular damage in rats fed on irradiated diets 705

Kutty M N
see Mohamed M Peer 67

Lazarus R J
On the length-weight relationship of the little-known lesser spiny eel, *Macrognathus aculeatus* (Bloch) (Pisces: Mastacembelidae) 423
Lemalatha M
Trypanosoma evansi infection and the influence of some hormones on haematological indices in albino rats 463

Mani A
see Rao Ch Venkata 397
Manna G K
Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera) 135
Marian M Peter
see Pandian T J 289
see Pandian T J 387
see Pandian T J 641
Maruthamuthu S
Some observations on the biology of periwinkle *Littorina scabra scabra* (Linne 1758) 269
Mathavan S
see Christopher M S M 303
Medda P K
see Ghosh D 181
Mehrotra K N
see Sriramulu M 147
see Gujar G T 689
Miglani Gurbachan S
Induction and genotypic verification of recombinants in males of *Drosophila melanogaster* 587
Mini A
Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) 379
Mohamed M Peer
Influence of ambient oxygen and random swimming activity on metabolic rates and quotients in the freshwater mullet *Rhinomugil corsula* (Hamilton) 67
Mohan S
see Muthukrishnan J 411
Mohideen H Md
see Baskaran R 59
see Raveendran S 567
Mukherji A P
see Venkatesh B 457
Mukhopadhyay P K
see Venkatesh B 457
Munuswamy N
Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca) 171
Murugesan A G
see Haniffa M A 155
Muthukrishnan J
Effect of temperature and metamorphic index on acid phosphatase activity of *Rana tigrina* 411

Nair K S S
The teak defoliator, *Hyblaea puera*: Defoliation dynamics and evidences for short-range migration of moths 7
Narayanan M
Occurrence of gastric absorption in the cichlid fish, *Sarotherodon mossambicus* 685
Natarajan R
see Ajmalkhan S 515
Nautiyal Prakash
Discharge-time method. A new technique for sampling plankton in fluvial systems, with a note on the modified plankton sampling device 751

Pal Dhananjay
see Chatterjee Shilla 223
Pandian T J
Production and utilization of frogs: an ecological view 289
Prediction of absorption efficiency from food nitrogen in amphibians 387
Prediction of assimilation efficiency of lepidopterans 641
Pandit Tapan
see Homechaudhuri Sumit 617
Parshad V R
Effects of ecological factors on the performance of rodenticides in sugar cane 729
Parulekar A H
Effect of mining activities on the clam fisheries and bottom fauna of Goa estuaries 325
Pathak P H
Variation in the reproductive capacity of *Earias vittella* (F.) (Lepidoptera: Noctuidae) following antennectomy or alactomy in males or wing loss in both sexes 613
Pawar C S
Heliothis species and their natural enemies, with their potential for biological control 695
Poddar Subhas
see Homechaudhuri Sumit 617
Porchelvi M
see Haniffa M A 155
Prabhu V K K
see Mini A 379

Prakash Ishwar
  see Idris Mohd 89
Prasad R S
  see Daniel B Ayyachamy 475
  see Sasikumar P S 549
Prema Suryanarayanan
  see Sasikumar P S 549

Raghavan R
  see Joel D R 437
Rajendran K
  see Ajmalkhan S 515
Raj P J Sanjeeva
  see Joel D R 437
Raman K V Anantha
  Distribution and abundance of prawns in the freshwater habitats of Bangalore, South India 77
Ranganath H A
  Competition studies in Drosophila, Relative fitness and adaptedness of six closely related species 199
  see Ushakumari A 557
Rao A Bhaskar
  see Kulkarni G K 723
Rao Appaswamy M
  see Kahwal B B 263
Rao C A Nageswara
  Reproductive cycle of the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) from Visakhapatnam coast 1
  Changes in the Haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura) 713
Rao Ch Venkata
  Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita* 397
Rao D G V Prasada
  see Devi V Uma 539
Rao K Hanumantha
  see Rao C A Nageswara 1
  see Rao C A Nageswara 713
Rao K R S Sambasiva
  Impact of phenthoate in continuous flow and static media on the respiratory enzymes of the murrel, *Channa punctatus* (Bloch) 719
Rao K V Ramana
  see Reddy M Srinivasulu 525
  see Reddy M Srinivasulu 533
  see Rao K R S Sambasiva 719
Rao L Narasimha
  see Lemalatha M 463
Rao P Kameswara
  see Rao Ch Venkata 397
Rao P R
  see Triveni A 255
Rao Y Prabhakara
  see Devi V Uma 539
Rathore N S
  see Roonwal M L 191
Raveendran S
  Visual capacity of marine teleost *Nemipterus japonicus* (Bloch) 567
Reddi P Sita Rami
  see Lazarus R J 423
Reddy M Srinivasulu
  In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure 525
  Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure 533
Reddy S Ravichandra
  see Raman K V Anantha 77
Renukaradhya K M
  Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) 103
Roonwal M L
  Wings and wing-microsculpturing in the termite family Indotermitidae (*Indotermes*, Isoptera), and their bearing on phylogeny 191

Saini S Manjit
  see Toor H S 429
Saklani A S
  see Nautiyal Prakash 751
Saksena D N
  Polymorphosis in a brachionid rotifer, *Brachionus quadridentatus* Hermann from Morar channel, Gwalior (India) 365
Sanjayan K P
  see Ananthakrishnan T N 117
Sarkar S K
  Composition and variations in the abundance of zooplankton in the Hooghly estuary, West Bengal, India 125
Sasikumar P S
  Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India 549
Senthamizhselvan M
  see Muthukrishnan J 411
Shahi K P
  Effect of certain experimental regimens on mating behaviour and the impact of nutritional difference between copulating males and females on the reproductive programming in *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae) 677

Shetty P S
  see Dechamma P A 45
Shyama Sundari K
  see Rao C A Nageswara 1
  see Rao C A Nageswara 713
Singhal R N
  The physico-chemical environment and the plankton of managed ponds in Haryana, India 353

Singh B N
  see Sarkar S K 125
Singh H R
  see Nautiyal Prakash 751
Singh Rajbir
  Ontogeny of play behaviour in free-ranging rhesus macaques (*Macaca mulatta*) 605
Sinha M
  Functional morphology, anatomy and histology of the digestive organs of the catfish, *Plotosus canius* Hamilton 23
Sokolov V E
  see Balakrishnan M 667
Soni B K
  see Mohd Idris 417
Spencer Kenneth A
  Agromyzidae (Diptera) in Thailand: new species, revisionary notes and new records 487
Sriramulu M
  Effect of diflubenzuron on DNA content during pupal development in *Corcyra cephalonica* Stainton 147
Subramoniam T
  see Munuswamy N 171
Sudheendrakumar V V
  see Nair K S S 7
Sukumaran N
  Oxygen consumption, carbon dioxide output and nitrogen excretion in cichlid fish, *Oreochromis mossambicus* (Peters), with special reference to swimming speed 215

Thangaraj T
  see Jeyaraj R 739
Thapliyal J P
  see Gupta B B P 403
Thomas Cheriyan
  see Daniel B Ayyachamy 475
  see Sasikumar P S 549
Toor H S
  Feeding ecology of the large grey babbler *Turdoides malcolmi* 429
Triveni A
  Tissue distribution and characterization of LDH isozymes in two fishes of the order cypriniformes 255

Ushakumari A
  Adaptedness of five closely related strains of *Drosophila* to media containing molasses or fructose 557

Vajranabhaiah S N
  see Krishnamoorthy R V 341
Varghese T J
  see Renukaradhya K M 103
  see Vijayan M M 371
Vasuki C A
  see Jeyaraj R 739
Velayudhan R
  Pollination potential of thrips (Insecta: Thysanoptera) in some Solanaceous plants 109
  see Annadurai R S 745
Venkataraman K
  Influence of various concentrations of rice bran with tap water and pond water on the longevity, egg production and body size of *Daphnia similis* Claus (Crustacea: Cladocera) 163
  Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* Claus and *Daphnia cephalata* King (Crustacea: Cladocera) under laboratory conditions 247
  Anisops bouvieri predation and advantages of cephalic expansion in *Daphnia cephalata* King and the impact of predation on *Daphnia similis* Claus under laboratory conditions 509
Venkatesh B
  Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets 457
Vijayan M M
  Effect of artificial aeration on the growth and survival of Indian major carps 371
Vindhya Mohindra
  see Miglani Gurbachan S 587
Viswanathan Kasi
  Host specificity of rice green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål) 227